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Recombinant DNA: now is the time for Congress to act

EVER since scientists first suggested that the uncertainties of recombinant DNA research made it advisable to agree on guidelines for containment levels, there has been no logical reason why research in private industry should be carried out under different conditions from university laboratories. After all, the essential concern has been over the potential health risks associated with this research, and such dangers do not differ from one context to another. Nor should one assume that industrial scientists are any more scrupulous than their academic colleagues.

There has thus always been a strong case for extending the guidelines established by the National Institutes of Health for federally-funded research workers in the US to cover the private sector as well (a member of the NIH's Recombinant DNA Advisory Committee recently called it "one of the most bizarre situations in the history of US science and technology" that this was not already the case). The essential question has not been whether this should be done, but how. And it has been the suggested answers to the 'how' questions, embroidering in various ways what might otherwise appear to be a relatively simple legislative concept, that have brought doom to a succession of congressional initiatives.

In the meantime, the process of developing, administering and revising the NIH guidelines has been proceeding relatively smoothly. Many scientists continue to believe that, even in their current form, the guidelines are too restrictive, and to baulk at the extra paper work involved. Others admit, however, that while some risk experiments have tended to confirm certain types of risks as being relatively small, others have indicated hitherto unanticipated potential hazards (such as the ability of DNA strands to induce tumours in laboratory animals). And that as long as these uncertainties remain, which itself is unpredictable, then some type of regulatory framework is necessary to cope with them.

For non-federally funded research, however, the picture remains confused. In the absence of new legislation, attempts have been made to fit the control of research using recombinant DNA techniques into existing legislative and institutional responsibilities. But the novelty of the hazards means that the fit has been an uncomfortable, and possibly untenable one. Thus Mr Califano, Secretary for Health, Education and Welfare, has said that he is reluctant to use powers granted to him under the Public Health Service Act, as some have been suggesting, as he is not sure whether the act is appropriate to the DNA case. In turn the Food and Drug Administration has decided that it probably lacks the legal power to require that all research leading to products submitted for licensing be carried out under the NIH guidelines as it had earlier been proposing to do.

The director of NIH has agreed that companies which express a willingness to comply voluntarily with the existing guidelines would be able to register with the NIH to have their research procedures approved. But the NIH is not, and has no desire to be, a regulatory agency.

(The Environmental Protection Agency and the Department of Labour's Occupational Safety and Health Administration have both kept relatively quiet on the issue, but in both cases there seems to be uncertainty over how to proceed with what remains a conjectural hazard.)

In the circumstances, there now seems a good chance that a new legislative initiative in the US Congress would be welcomed by many sides in the debate. To members of the Administration and federal institutions it would provide a clear delineation of roles and responsibilities in what will otherwise remain a murky situation. To private industry, although opposed in principle to government regulation, it should provide considerably more credibility than current promises to comply voluntarily with the NIH guidelines. And to environmentalists, public interest groups and labour unions, it would signal that private industry was not being given privileged status in the DNA debate (while also providing a channel, if minimal, for public participation in the type of debates that are constantly being urged on the future direction of new technologies with important social implications).

As with the previous legislative attempts, of course, the most controversial issues will inevitably be on the procedural, rather than the technical, aspects of complying with the guidelines. The fact that industry has already indicated that it is willing to comply voluntarily with the guidelines—and the extent to which industry spokesmen have gone to confirm that even at present, private research workers are following recommended physical and biological containment levels—shows that the inclusion of this aspect in new legislation should not provide too much of a problem. The sticky issues, however, will be over the appropriate forms of accountability and responsibility—over who should have access to the decision-making process, on what basis, and what should be done about data needed for the purposes of regulation but which a firm considers to contain information that would be valuable to a commercial competitor.

These are all political questions. At present they are being resolved by default by conventional means, with the decision-making agenda drawn up and controlled by those who stand to gain financially from a successful outcome to the research. It is up to those who, we hope, are now considering possible forms for legislation to decide whether this is the way they wish it to be, or whether to take bolder steps (such as involving trade union representatives in the decision-making process at a local level). It will take a political desire for change to carry it through, but a desire with which Mr Califano has already indicated his sympathy by expanding public interest representation of the RAC last December. RAC has, like Britain's Genetic Manipulation Advisory Group, been held up as a model pointing to the future direction of public participation in technological decision-making. Despite current limitations, we feel that this direction is the right one. And that regulation extending the NIH guidelines to industry should reflect this conviction. □



Computers lose on the swings and the roundabouts

Tight university research budgets are contributing to a shortage of computer science graduates in the US. But concern is also growing about the social effects of automation.

David Dickson reports

NEXT Saturday, half a million members of the Communication Workers of America are holding a national "Job Pressures Day" to protest at the way in which automation is dehumanising working conditions. No one will stop work, the intention is to draw public attention to the issue through informational picketing and other activities under the general slogan "we are people not machines".

The action is claimed to be unprecedented for a major US union, most of which keep strictly to the conventional issues of wages and working hours. But it heralds a trend within the US labour movement which, fuelled by evidence that automation may finally be resulting in the unemployment previously predicted but unrealised, promises to grow substantially over the next few years.

Even without these new complaints about working conditions, the manpower implications of computers—a subject of hot debate in the late 1950s and 1960s, but one that subsided as economic prosperity cushioned any significant impact—are once again causing serious concern in policy-making circles.

On the one hand, support is declining for experimental computer science in US universities, and attracting good staff and students has become a problem. As a result, there are not enough qualified graduates for industry's needs. Many computer companies now face major difficulties in recruiting staff and have expressed their concern to the Office of Science and Technology Policy.

A report published last month by an advisory committee to the National Science Foundation says that as a result of funding difficulties, computer science research is now in a "critical" situation. University programmes are declining, just when the size and quality of research and training should be increasing to meet industry's needs. The report points out that, with obsolete equipment and inadequate finance, many of the best staff are now being recruited away from universities, whose research laboratories were originally responsible for such central innovations as time-sharing techniques, virtual memory systems and file protection mechanisms.

A particular concern, the committee says, is the fall in the number of computer science doctorates in the past

two years. "Because of its leverage in stimulating research, retaining faculty and capturing the interest of superior students, the most important requirement is the establishment and maintenance of outstanding university research facilities," it says. This could be achieved by injecting as little as \$15 million a year, based on five capital grants of \$2 million to separate institutions, plus running costs for each for the following five years. OSTP is now trying, with the support of industry, to persuade the Office of Management and Budget that this will be money well spent.

But while the management side of industry is complaining about a shortage of qualified recruits, labour is concerned about those who will lose their jobs as a result of automation. Until recently, the conventional wisdom was that unemployment was outweighed by the extra jobs created through increased prosperity. Now, with a stagnant economy plus the rapid incursion of automation into previously untouched areas such as office work, there are doubts about this equation.

A typical survey carried out by the Society of Manufacturing Engineers predicted recently that 20% of the labour directly involved in the final assembly of automobiles will be replaced by machines—such as robot

welders—by 1985, and 50% by 1995.

Similarly, a Bureau of Labour Statistics report on telecommunications technology concludes that "the anticipated strong demand for communications will probably modify the adverse effects on employment, but can no longer offset them. As a result, employment is expected to decrease slowly into the mid-1980s, significantly altering job skills and occupational composition."

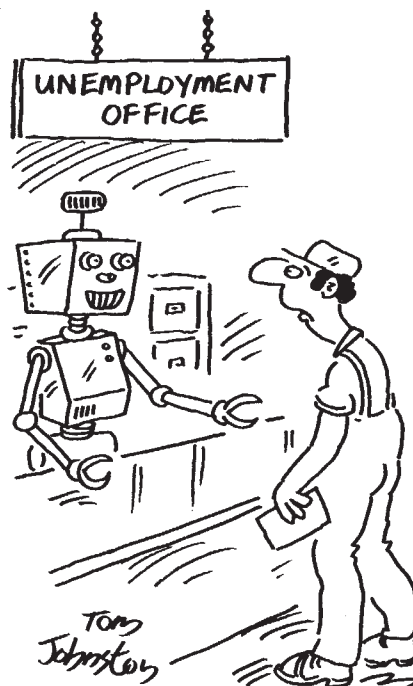
Such automation seems likely to result in a "substantial permanent sector of unemployment", as a result of society's inability to cope with the effects of new technology, predicts Robert T. Lund, a senior research associate at MIT's Centre for Policy Alternatives. Part of the problem, he suggests, is that the 1950s automation scare which failed to materialise has led to a false sense of security.

Some indication of the size of the problem may be given when the Office of Technology Assessment produces the results of a survey it is conducting as part of a broader project on the social impact of data processing systems, with a view to seeing whether any Congressional action may be needed. "It is certainly an issue that deserves more attention than it has been getting," says Mr Stephen Doyle, group manager for telecommunications at OTA.

Meanwhile there is discontent among those who are at the receiving end of the new technology. A survey conducted by the University of Michigan has just reported that, for the first time, there was a decline in the national level of job satisfaction, as measured between 1973 and 1977. The survey of 1,515 workers found that 36% felt their skills were underused, and 32% that they were "overeducated". There was a "slight but significant" drop in overall job satisfaction.

The unions are already beginning to react, largely in response to rank-and-file pressure. In addition to next week's WA demonstration, members of the United Auto Workers made strong demands at a recent conference for tougher terms on retraining, monitoring technical change and giving early notice of automation plans.

Mr Glenn E. Watts, CWA president, goes further. In a recent article he said that his union believes major technological advances should be held in check until a human impact study has been made, just as environmental impact studies are carried out in other circumstances. "If some workers today are bored and alienated, the degrading of work itself must be recognised as part of the problem." □



FDA backs off attempt to control private DNA research

THE US Food and Drug Administration has concluded that it probably lacks the legal authority to regulate recombinant DNA research in private industry, as it had previously announced its intention to do following a directive from Health Secretary Joseph A Califano last December.

Instead, the FDA plans to encourage companies to comply voluntarily with the guidelines that have been established by the National Institutes of Health for federally-funded research. The NIH is itself recommending some amendments to the guidelines to make them more acceptable to industry.

Two weeks ago, however, the NIH's Recombinant DNA Advisory Committee passed a resolution recommending that private industry be legally required to observe the guidelines. It is now up to Mr Califano to decide whether a scheme of voluntary compliance is adequate, or whether to seek another route for regulation—possibly involving a new legislative initiative.

Following the failure of numerous attempts in the past to extend the NIH guidelines to industry, Dr Fredrickson, director of NIH, announced last year that companies would be able to register with the NIH—if they wished—to have containment levels for proposed experiments approved.

So far, however, companies have been reluctant to register, fearing that compliance with some sections of the guidelines would require them to divulge commercially-sensitive information. At least one company—Genentech, in San Francisco—is now conducting experiments with 60 litres of culture which would have required submitting full details to the RAC for a special exemption from prohibition under current NIH procedures.

The NIH is now proposing to add a new section to the guidelines to meet

such fears. For example, although academic scientists wishing to have a new host-vector system approved by the director of NIH make available full details of the system and its proposed use, this would be waived for private companies, who say it could require disclosing commercial secrets.

Under the proposed addition to the guidelines, private companies would set up their own institutional biohazard committees. But members of such committees would be exempt from the requirement that they should not be involved in approving a project in which they had a financial interest.

The NIH has also gone to some lengths to establish that it would be a criminal act for any member of the RAC, as "temporary government employees", to divulge confidential commercial information submitted to the committee, and that this information would therefore be immune to the Freedom of Information Act.

At a meeting with HEW general counsel Mr Peter Libassi last week, industry representatives said they were reasonably happy with the new proposals as a basis for voluntary compliance. Public interest and labour representatives, however, were adamant that a voluntary system would not work and that some form of legal back-up was necessary. They also urged that the Environmental Protection Agency and the Occupational Safety and Health Administration take a more active role.

The position on legal controls has been complicated by the FDA's decision. Last December, coinciding with the publication of a revised version of the guidelines and following a directive from Mr Califano, the FDA published a "notice of intent" to introduce regulations. These would require a firm submitting a product



Califano: he must decide

for licensing by the FDA to demonstrate that any recombinant DNA research carried out in connection with the product had been done in accordance with the NIH guidelines.

Pharmaceutical companies, however, challenged whether the FDA had the authority to do this under the terms of the federal Food Drug and Cosmetic Act, claiming that the agency's authority was restricted to clinical trials and marketing.

Dr C. W. Pettinga, executive vice-president of Eli Lilly and Company, commented: "In our view the proposal must be carefully evaluated because it could evolve into the regulation of basic research, an undesirable circumstance from a public policy standpoint and clearly not warranted in view of the experience with recombinant DNA technology."

No public reply has yet been made by the FDA. But in the light of these and similar comments, officials in the agency confirmed last week that the proposal to introduce new regulations had been dropped and that support was being recommended for the voluntary compliance scheme as being proposed by the NIH.

Dr Fredrickson is now expected to forward these comments, as well as both the NIH's proposals and the RAC resolution, to Mr Califano—who will have to decide how to take things from there.

David Dickson

Plan for early warning on social effects of biomedical advances

THE National Commission for the Protection of Human Subjects, a body set up in 1974 to study the ethical, social and legal implications of advances in biomedical and behavioural research and technology, has recommended setting up an advisory commission "to anticipate the probable effects of research and technological advances for individuals and society, and to stimulate public participation in decision-making".

This recommendation has arisen from a special study conducted by the commission, whose conclusions were published in the *Federal Register* last

week. The commission says that the results of the study showed both a perceived need for a programme to assess the social impact of technology, and a need to facilitate public information and public participation in research and technological innovations and the policy decisions that result.

"These findings suggest that a mechanism should be established to monitor and evaluate innovations and to provide an early warning system in which the probable effect of innovations in biomedical and behavioural research and technology can be assessed publicly, prior to develop-

ment of widespread dissemination", the commission says. Existing entities, such as those attached to the National Academy of Sciences or the National Institutes of Health, served narrower constituencies and goals, and the independence and broader mandate of a new body were needed, it says.

"The commission should not be dominated by health professionals, for its main purpose would be to facilitate widespread debate involving all segments of society in the ethical and policy issues that affect all people and about which diverse views should be heard."

California report pinpoints hazards in layout of Three Mile Island control room

IN early May, California's governor Edmund G. Brown Jr called for a national moratorium on construction of nuclear power plants and declared himself to be "at the forefront of the antinuclear movement". Some political observers have suggested that his actions represent a new expression of his presidential ambitions, but the governor also seems to be taking seriously a report prepared by his staff that condemns the state of 'human factors engineering' in the nuclear industry. In a letter to Nuclear Regulatory Commission (NRC) chairman Joseph M. Hendrie, Brown wrote: "I am informed that the design of nuclear power plant control rooms may represent a significant safety hazard".

A copy of this staff report, together with some of the supporting documentation, has become available to *Nature*. The report was prepared by Wilson Clark, Assistant to the Governor for Issues and Planning, and three assistants, two of whom have experience in nuclear engineering. It concludes that many nuclear power plants "are poorly instrumented and designed for adequate reactor operator control . . . Modern nuclear technology is harnessed by antiquated control technology which represents a clear and present hazard."

Clark summarised the report's conclusions in a letter to the chairman of the Presidential Commission on the Accident at Three Mile Island, John G. Kemeny. He cited the NRC's preliminary evaluation of the Three Mile Island accident as a vindication of his belief that poor design for human operation were "a leading, if not the leading factor, in the near-disaster at Three Mile Island." He catalogued three main hazards covered in his report that bear on Three Mile Island:

- poor layout, making plant operation difficult and dangerous;
- inadequate display of control room information; and
- confusing and incomplete operational procedures which lengthen operator responses, decreasing reliability.

Clark's point has been made before, but the issue generated little interest until the series of interrelated human and instrumentation errors at Three Mile Island. The famous Reactor Safety Study, WASH-1400, which gave the nuclear industry a generally clean bill of health, criticised the design of controls and displays and their arrangement on operator panels. Later, a task force of the Electric Power Research Institute (EPRI) expressed similar criticism and recommended that EPRI review the problem. The review was

conducted by the Lockheed Missiles and Space Company and published in late 1976. Although the conclusions of the Lockheed/EPRI report were generally low key, the illustrations were devastating. With such understatement as "the study findings paint a rather negative picture" the report catalogues a substantial list of design faults that could directly affect an operator's ability to react quickly in an emergency. These include:

- massive arrays of identical control/display units with no clearly identified subpanel grouping;
- control levers so large that individual control panels expand to enormous size;
- separation of related panels into two areas so that two operators are required to coordinate their actions even when separated by as much as 50 feet; and
- placement of gauges so high that an operator cannot read them without standing on a footstool, or on a wall opposite the location of related control levers.

Even such a simple matter as the number, placement and ease of replacing signal lamps can have serious consequences. The Lockheed/EPRI study found that "indicator reliability is a problem and there are a surprising number of burned-out, single lamp indicators at any given time" in the reactors surveyed. Warning lights are so numerous that many operators become cavalier about 'nuisance alarms'. And to replace burned-out lamps operators sometimes stand precariously on a control panel.

The Clark report shows how prophetic this passage of the 1976 study was to be. In March 1978, an operator dropped a light bulb into an open con-

trol panel at California's Rancho Seco plant, while trying to fix a burned out indicator. The resulting short circuit caused the reactor to shut down, knocked out two-thirds of its temperature, pressure, flow and level signals, and cut off auxiliary feedwater for seven minutes. Clark concludes: "Had other safety systems not performed adequately, this transient could have been similar to the accident at Three Mile Island, which experienced a loss of auxiliary feedwater cooling for only eight minutes."

Governor Brown's team also cited a study of the Zion nuclear power plant by Alan Swain in 1975, for the NRC. According to the Clark report, the owner of the Zion plant had been able to suppress distribution of the document, which had found in a 'talk-through' of procedures to use in a serious accident, that "even experienced operators had some difficulty in locating particular controls and displays."

It remains to be seen what action will be taken to improve the confusing array of instruments, dials and switches facing reactor operators, but the NRC has at least acknowledged the problem. A recent staff report on reactors designed by Babcock and Wilcox (who designed the plant at Three Mile Island) states: "Human factors engineering has not been sufficiently emphasised in the design and layout of the control rooms. The location of instruments and controls in many power plants often increases the likelihood of operator error or, at the least, impedes the operator in efficiently carrying out the normal, abnormal, and emergency actions required of him."

John Douglas

US to study health effects of accident

THE US Department of Health, Education and Welfare announced last week that it is to carry out a number of surveys of the possible health effects on those who were exposed to radiation as a result of the nuclear accident two months ago at the Three Mile Island nuclear power plant in Pennsylvania.

One survey will cover pregnant women living within 10 miles of the plant, who will receive regular health checks throughout their pregnancy. Their children will be monitored for the first few months after birth. The National Institute of Mental Health will carry out a survey of a representative group of people "subject to the strain of the accident", and a

further study will examine the health of the workers at the plant.

In addition there will be a general survey of the health of 50,000 residents living within five miles of the plant. "It's just common sense to do this. There is no reason for alarm. I said some time ago that we would study the population, and that we would particular focus on pregnant women", Health Secretary Joseph Califano said last week. The survey will be financed by the Center for Disease Control and the National Cancer Institute. State officials hope to continue the study on a year-to-year basis for up to 20 years.

David Dickson

Support grows for rival conference on development

SEVERAL hundred non-governmental organisations have already expressed interest in participating in an alternative conference which is being planned to take place in parallel with the United Nations Conference on Science and Technology for Development in Vienna at the end of August.

Unlike the official conference, which will concentrate on political mechanisms for stimulating the contributions of science and technology to development, the alternative conference will consider specific topics for action. High on the preliminary agenda are plenary sessions on nuclear energy in Third World countries, environmental aspects of development, the role of multinational corporations, and the implications of the arms race. These sessions will take place in Vienna's Kongresshaus.

A number of working groups are also planned, to run for the full ten days of the conference (August 19 to 29) with the aim of producing specific proposals for action. In particular, there will be discussions about information and financial mechanisms that might be established to support the work of non-governmental organisations after the conference. "This is one of the most important things that we can do at Vienna", says Dr Karim Ahamed of the Natural Resources Defense Council in New York, chairman of the conference organising committee.

"We want to see the extent to which non-governmental organisations can, for example, stimulate technology assessment activities in Third World countries which could be brought to bear on government efforts. And we also want to find out what private and public sources of funding are available to support these efforts."

The organising committee, which has a planning board made up of 14 international organisations, is already preparing an extensive document commenting on the proposals for action that will be discussed at the official conference, and pointing out areas—such as the need for appropriate technologies, or the role of women in development—which it feels have been neglected in official discussions.

"One particular goal will be to see if we can create mechanisms for monitoring what happens after UNCSTD, and exposing it to public view in an effort to make governments keep up to their promises," Mr Ward Morehouse, another member of the committee, said. □

Max Planck Society sets up three new research institutes—and cuts off support for others

THE Max Planck Gesellschaft (MPG), which spends DM750 million a year on research in West Germany, has started to renew and restructure several of its 50 institutes. It is following a tradition of, from time to time, taking up new fields in exchange for others that have matured or have not developed according to the MPG's expectations.

Last March, the MPG Senate, for the first time in five years, approved the setting up of new institutes (MPI) and gave the green light for three of them straightaway: an MPI of Quantum Optics (Directors: Professors Karl-Ludwig Kompa, Herbert Walter and Siegbert Witkowski); an MPI of Psycholinguistics (Director: Professor Dr Willem J. M. Levelt); and an MPI of International and Foreign Social Legislation (Director: Professor Dr Hans F. Zacher).

In addition, one or two project groups working closely with clinical establishments (eg in universities) are to be set up to augment medical research within MPG. The content and orientation of their research is not yet decided, but it is likely that cancer research will be among the new fields.

The decisions to set up the three new institutes have been taken after several years of successful work by project groups. (This is an important condition within the MPG for the establishment of a new institute.) The new MPI of Quantum Optics is building on the work of the project group for laser research, which itself was hived off four years ago from the MPI of Plasma Physics (IPP). And the future Institute of Psycholinguistics, which will research the structure and use of natural language is building on work already done at the Netherlands University of Nijmegen. The decision to site the latter at Nijmegen is seen as an example of MPG's emphasis on European cooperation. The MPG, which is financed jointly by the federal research ministry and the *Länder*, hopes to fund its new activities from higher growth rates in the budget (6–8% per annum instead of an average of about 3% during recent years of stagnation).

The MPG has also decided to close down work at two of its institutes. Terminating work in a particular field or closing an entire institute is usually done when similar research is being carried out in universities and other research establishments (and mostly when the head of the institute retires).

Thus Professor Jürgen Aschoff's research at the MPI of Behavioural Physiology runs out with his retirement because biorhythms are being researched simultaneously at other places. (His famous 'bunker' will continue to be available to researchers!) For similar reasons the work on nuclear physics at the MPI of Chemistry in Mainz is to run out and be replaced by the new main field of geochemistry.

On average, over the past 10 years, one institute or department within the MPG has been closed or transferred to another agency each year. In the majority of cases the public has been aware of little of the concomitant tensions or problems. Not so, however, in the case of the institute founded in 1970 by Carl Friedrich von Weizsäcker in Starnberg with the ambitious title of Max Planck Institute of Research into the Living Conditions of the Scientific-Technological World. The founding of the institute created a stir in particular because of the all-embracing formulation of its brief (which prompted *Der Spiegel* on one occasion to talk of a 'Faustian' project); the overt attempt by industrialists to influence its policy; and the appointment of the neo-Marxist Jürgen Habermas as co-director.

Doubts later grew about the efficiency of the institute, which seemed to be concentrating on isolated projects. Even though one or two noteworthy pieces of work were done, the commissions, set up as a rule four years before the retirement of an institute head, started to discuss the end of the institute in its existing form. After intensive discussions the MPG Senate decided not to continue the work of Herr von Weizsäcker after his retirement "since there is no one to replace him".

Instead, a new MPI of Social Sciences is being set up, which will have four departments and according to Professor Reimar Lüst, President of the MPG, who was talking during the MPG's general meeting in Mainz last month, "is advancing no sweeping claim for the social sciences, any more than would an Institute of Physics or Biology in our society". Ralf Dahrendorf, at present Director of the London School of Economics and Political Science, was appointed as an additional director with Jürgen Habermas. Two more departments are envisaged for a political scientist and a psychologist.

Klaus Höpfner

Soviet scientists visit UK and 'sell' technology

"SCIENCE is universal because it strives after universal truths that can be universally verified. Scientists have no option therefore but to collaborate and have done so successfully even when political relations are bad. But technology is different; because it is 'sensitive'."

So said Professor J. Ashworth, Senior Scientific Adviser to the Cabinet Office at the opening last week of the "Days of Soviet Science and Technology" organised in association with the USSR National Exhibition at Earl's Court, London. By this criterion, it might be deduced, both from the exhibition itself and from the composition of the scientific "touring team", that détente is alive and flourishing. For both are strongly orientated towards technology.

For the exhibition, this may be inevitable; a Soyuz space-capsule or non-polluting oil refinery is much easier to demonstrate to the public than the latest developments in quantum mechanics. Even the announced "massive" participation of the Soviet Academy of Sciences, apart of course for the baby mammoth, reduces to a few technological exhibits—some special industrial alloys, a method of shaping high-precision tools from graphite and then converting them to diamond, and a laser landing system for aircraft which, Academician Basov, the leader of the scientific delegation urged, would be particularly suitable in the British climate.

Basov, who holds a Nobel Prize in physics for his work on lasers, has become, in the context of this tour, virtually a salesman for Soviet laser technology. Even addressing the Royal Society, his subject matter was more practical than theoretical—laser welding, laser precision systems for wire manufacture, laser coagulation for eye-surgery, a laser-based 2,000-line TV set in the "nearest future", as well as the prospects of lasers in the 10^5 – 10^9 J range and a "very promising" hybrid reactor with a "thermonuclear target surrounded by fissile material". The main problem with the latter, he said, will be the "service life but ideologically we are prepared for further developments".

Equally a "salesman" but with longer aims in view is Professor Andrei Kapitsa, whose speciality is remote sensing from space. This he sees as a major field of potential cooperation, particularly in "global problems" such as pollution. "Even a country as large as the Soviet Union cannot save the problem of air pollution", he said. "This can only be done on an international basis." Then, too, there are problems of induced climatic change.

A major irrigation or rain-making initiative by one country could have disastrous consequences for another, if there is no international consultation. According to Kapitsa, however, Soviet planners "don't yet use space data directly, but only at the second or third remove." Science, he said, "is always two steps ahead, giving the economy a tug!"

The "Days of Soviet Science and Technology" are presumably designed to give a similar "tug" to British-Soviet economic cooperation. "Economic cooperation goes very closely with such things as know-how", Kapitsa explained. "Know-how is sold, and know-how is science".

Several members of the delegation, however, saw the "Days" rather as a useful chance of pursuing their own research. The three Medical Academicians, Kochetkov, Loginov and Puchkovskaya all spoke in terms of existing cooperation. So did Academician I. M. Kolotryrkin, who explained that in his particular field—metal corrosion—the UK and USSR have already achieved a *de facto* division of labour. "Britain is doing some very interesting work on the metallurgical side", he said, "stress corrosion, cracking and so on. In the Soviet Union we use mainly a chemical and radiochemical approach. This is a genuine sharing of effort".

For Professor F. V. Sapozhnikov, however, diverging priorities between the UK and the USSR might well have made fruitful discussions difficult. For as Deputy Minister of Power and Electrification, he is committed to a major nuclear power programme, which includes not only power generation, but even the production of hot water for district heating from small reactors sited on the outskirts of cities. (It is considered sufficient for

safety if a zone 3 km in radius round the reactor is kept free from residential use, although it may be used for agriculture, industry or leisure purposes).

The commitment to nuclear energy was well represented at Earl's Court; exhibits included a scale model of the BN-600 water-water reactor intended for the third set of the Beloyarsk power station and a diorama of the South Ukrainian power complex, which will be based on $4 \times 1,000$ MW nuclear generating sets, with a 1,800 MW hydroelectric auxiliary plant for peak hours and a 380 MW pumped storage system.

Sapozhnikov did not see Britain's current disenchantment with nuclear power as any barrier to cooperation. "We pay a great deal of attention to UK experience", he said. "We have enjoyed a good cooperation programme for several years now, and it has worked quite successfully".

Such remonstrances have in general been fairly infrequent. Apart from the constant stress that exchange benefits the UK as much as it does the Soviet Union, there has been little emphasis on "misunderstandings" and much on mutual benefits and the building of what Basov called "a bridge of confidence" between Soviet and British scientists.

"Our main task is to learn what others have done and to show what we have done", Basov explained. Then, doubtless not unaware of the possé of demonstrators who had dogged the delegation with their banners demanding the release of Orlov, Shcharanskii and Kovalev, he added, "We respect and treat with understanding the customs of your country. As the Russian proverb says 'When you arrive at a strange monastery, don't try to induce your own rules there';"

Vera Rich

Soviet progress in wind and solar power

SOVIET solar energy enthusiasts may one day give a new meaning to the term industrial plant. Recently Dimitri Zhimerin, Deputy Chairman of the USSR State Committee for Science and Technology, said that "scientists believe that solar energy could be used for industrial applications if generators were developed imitating photosynthesis". So far, however, no-one has announced artificial photosynthesis as a viable method.

Zhimerin's statement, however, came in an otherwise factual account of research into solar and wind power in the Central Asian Steppes. In addition to solar heating for domestic use considerable progress, he said, has been

made in water desalination for state cattle farms. Here the breakeven point occurs when water would otherwise have to travel 35–40 km.

Extensive use of solar energy in these regions could, he said, save the Soviet economy 15–20 million tonnes of conventional fuel per year while wind energy, properly exploited, could produce 11×10^6 MW, 50 times the total capacity of all Soviet power stations in 1976. According to Zhimerin, the first wind powered electrical generator with a capacity of 100 kW was built in the Soviet Union in 1930. The experience gained with it has led to the development of "wind powered units, both mechanical and electrical". □

news in brief

CO₂ increased by 1.5 p.p.m. in atmosphere last year: The US National Oceanic and Atmospheric Administration reported that the average carbon dioxide concentrations in the earth's atmosphere rose to 335 parts per million last year, an increase of 1.5 parts per million from 1977, and of 21 parts per million—or 6.3%—since 1958. A spokesman for the agency said that the increase was principally due to increasing emissions of carbon dioxide from the burning of fossil fuel. Despite the increases, however, government scientists still believe that they have several years in which to decide whether the effects of the carbon dioxide build-up warrant restrictions on the burning of coal, to which the US is increasingly turning as an alternative fuel to oil.

Iran says no to nuclear power: The revolutionary government in Iran has cancelled all plans for nuclear power plants in the country including two contracts for plants already under construction. The head of Iran's atomic energy organisation, Fereidun Sahabi, said the contracts with France and Germany should be cancelled for "political, economic, social, human and technical reasons". The German plant is 80% complete but overspending of £1.5bn would mean that the final cost of £3.5bn for the plant would be more than twice the international standard. "It is true that work conditions in Iran are different than in Germany, but they are not that different", said Sahabi. An Iranian energy expert, formerly in favour of nuclear energy, has prepared an analysis showing that the plants cooling towers could be used as grain silos. The nuclear cancellations are only part of a massive reorganisation of Iran's economy.

The *Financial Times* estimates that multinational losses in Iran amount to £18.6bn in the civilian sector with an equal amount in the military sector, a level of loss "unprecedented in business world wide short of a natural disaster or a global war". US and UK firms have been hardest hit because of their heavy involvement in the sale of military equipment to the Shah. The rise of workers committees in the factories has been instrumental in the cancellation of many contracts.

Meteorologists approve world climate programme: The executive board of the World Meteorological Organisation at its 8th World Congress on 26 May passed a basic programme of research into the world's climate. The programme will collate data from WMO's World Weather Watch initiated eight years ago and data acquired from the climate programmes of member countries in order to assess climatic change and variability with special reference to predictability and human interference. This study will be carried with the International Council of Scientific Unions. In addition, a study of the impact of climate on human activities will be carried out in collaboration with the United Nations Environment Programme. Two other projects will be a new typhoon operational experiment in the Western Pacific with special attention to hydrological effects inland and a West African monsoon experiment. There will also be an increased emphasis on training programmes in Third World countries to be carried out with money from the UN Development Programme.

from Peter Collins in Geneva.

USSR asks US to halt space shuttle programme: The Soviet Union has asked the US to halt work on the space shuttle programme, which it sees as a possible threat to its own satellites, according to a report which appeared last week in the *New York Times*. The demand is said to

have come up during preliminary discussions on ways of eliminating the spread of so-called killer satellites, which are designed to locate and destroy other craft in orbit, such as surveillance and communications satellites.

The space shuttle, which has been developed by the National Aeronautics Administration for both civilian and military purposes, is due to be launched at the end of the year. It will be used to launch military satellites into orbit, but US officials deny that there is any intention of using it for anti-satellite activities. The Soviet Union has maintained, however, that the shuttle could threaten its satellites, and has therefore asked the US to halt the testing programme. US officials describe the request as "totally unacceptable" and feel that it rules out the possibility of an early agreement on killer satellites, which they had hoped to reach before the signing of the SALT treaty by President Carter and Mr Brezhnev in Vienna on 18 June.

Hundreds arrested, one killed in worldwide nuclear protests: A weekend of militant anti-nuclear activity was marred by the killing in Spain of Ladi de Estan Terrane, 24, who was shot in the head during a police charge on an anti-nuclear rally in Tudela in the Basque country. The killing provoked three hours of street fighting between demonstrators and police. Anti-nuclear demonstrations also drew large numbers of people in the Netherlands, Japan, the UK and West Germany. In Canada, five sky-divers jumped into forbidden territory at the world's largest nuclear plant under construction in Darlington, Ontario and 56 others were arrested at the plant owned by Ontario Hydro. In the US over 600 people were arrested in demonstrations at plants in 12 American states. In Inola, Oklahoma 339 demonstrators braved a biting rain to get arrested by climbing the fence guarding the construction site of the Black Fox nuclear power plant.

Test ban critic to head weapons research laboratory: The University of California announced last week that Dr Donald M. Kerr, currently a deputy assistant secretary in the Department of Energy, has been appointed the new director of the Los Alamos Scientific Laboratory in New Mexico, succeeding Dr Harold Agnew who resigned earlier this year. Dr Kerr worked at Los Alamos, which carried out a large proportion of the US basic research into new nuclear weapons, for ten years before moving to Washington. He became the focus of some controversy last year when he opposed President Carter's proposals for a five-year test ban treaty, claiming that a treaty of such length would raise questions about the reliability of weapons that were being stockpiled but not tested.

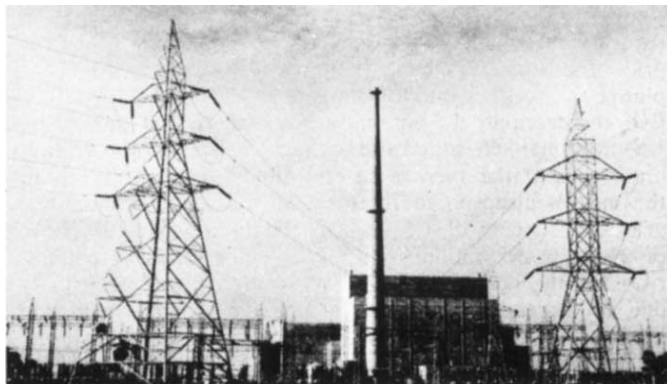
Mr Kerr's previous involvement in weapons research is likely to lead to renewed pressures at the University of California for the university to divest itself of the Los Alamos and Lawrence Livermore laboratories, which it manages on a contract basis for the Department of Energy. Last month, an advisory committee recommended to the Department of Energy that it should prepare contingency plans, in case the university pulls out.

Dungeness okay: The UK Health and Safety Executive has permitted the Central Electricity Generating Board to restart its Dungeness nuclear reactor. The reactor was shut down when micro-cracks were discovered in the welds of an expansion bellows. The CEGB claimed that the cracks were present at manufacture, have not increased during use and are not a hazard. Monitoring equipment has been installed as an additional precaution.

This nuclear power plant is contaminated.

India's demand for energy means it cannot be shut down

Tarapur, near Bombay.



Nuclear power needs to be regulated in the Third World just as much as in the West. But economic pressures in developing countries mean that safety may not always come first. Moreover, western insistence on regulation may be seen as an attempt to hamper Third World progress. Report by **Anil Agarwal**

THE accident at the Three Mile Island nuclear power plant has caused considerable concern in the developed countries. But very little has yet been said about the lessons to be learnt on the safety of nuclear reactors in the Third World. Even though volumes have been written about the problems of transferring sophisticated technology to developing countries, precious little has been published about the management and absorption problems posed specifically by the transfer of nuclear technologies to the Third World. The sole exception in this field is the burgeoning literature on the proliferation aspects of nuclear technology transfer.

In the past few years, rising costs and environmental protests have halted the growth of nuclear programmes in western countries. In 1977 almost no new reactors were ordered by the industrialised countries. During this time, it seemed as if the nuclear industry had begun to shift all its attention to Third World markets in particular, with French and West German companies indulging in aggressive selling openly aided by their governments. The prospects of large orders from Brazil, Iran and South Korea seemed to confirm this trend.

Since the revolution in Iran, however, Third World markets have received a serious blow. Iran has cancelled all the nuclear plants on order and according to unconfirmed reports, Iranian authorities—and for that matter, the Western nuclear suppliers too—may not be interested in completing the Bushehr nuclear power

plant which has reached an advanced stage of construction. Among the first set of people arrested by the Shah of Iran for mismanagement and embezzlement in his unsuccessful bid to satisfy the rising demands of his people was the then Chairman of the Atomic Energy Organisation of Iran. Since the overthrow of the Shah's government, several officials of the AEOI have left Iran leaving behind only a fledgling organisation.

Brazil's controversial nuclear programme too is running up against increasing domestic criticism largely because of escalating costs. The cost of Brazil's first power reactor, the 626 MW Angra-1, which is being constructed by Westinghouse, is now expected to be between \$850 million and \$1 billion compared to the initial estimates of \$218 million. The cost of the Brazilian-West German nuclear deal of 1975 under which Brazil was to be supplied with eight nuclear power plants of 1,320 MW each by the West German company Kraftwerk Union (KWU), has already risen from \$10 billion to \$13 billion. Industry is protesting at the projected increases in electricity tariffs. Meanwhile, US pressures have prevented the French sale of a plutonium reprocessing plant to Pakistan.

Third World nuclear programmes are, therefore, slowing down under a variety of economic and political pressures. But several developing countries nonetheless have or are planning to have significant nuclear programmes. South Korea ordered two new plants in 1978 and already has one reactor in operation, and two under construction. By the mid-1980s it expects to have eight nuclear power plants that will supply nearly one-third of its electrical needs. In nuclear terms, therefore, South Korea should become Asia's second Japan. Taiwan too is pressing ahead with its nuclear programme despite the supply problems posed by its current political difficulties. Taiwan's first power station consisting of two 636 MW units started operation in 1977 and two more large plants are expected to go into service

by the early 1980s.

The Pakistan Atomic Energy Commission has asked its government to sanction the purchase of a 600 MW nuclear plant. Turkey is expected to order its first plant from Sweden soon. Argentina already has one 319 MW power reactor in operation and another 600 MW reactor under construction. It is also expected to decide on the purchase of more reactors soon, possibly this month. Argentina is mainly considering tenders from Canada and Germany. Cuba plans to go ahead with a 440 MW nuclear reactor from USSR. Mexico has two reactors under construction which are expected to be completed by 1982. With the discovery of large oil reserves, Mexico may not be keen to go ahead with more reactors in the near future.

From all present indications, it seems that the incident of Three Mile Island has scarcely sent a ripple through those Third World countries which are keen to buy and build as many nuclear reactors as they can. Part of the reason for this behaviour is that nuclear programmes have come to be associated by developing countries with enormous political prestige. The efforts of western governments to control the spread of nuclear technologies are seen by many Third World governments as a crude attempt to monopolise a technology that is of considerable importance to the world. These discriminatory western pressures have helped to make nuclear power, as a senior IAEA official recently put it, "an immensely patriotic issue" in many developing countries and even in some developed ones like Japan. Under these circumstances, nuclear authorities in Third World countries will move very cautiously to accept safety-related arguments against nuclear power.

However, where safety *per se* may fail to move Third World nuclear authorities to ensure safest possible operation of nuclear projects, sheer economics will force them to consider all safety-related issues. Unlike the US, no developing country can suddenly afford to lose a billion dollar plant because of an accident. In some

countries, a large power plant can form as much as 10% of the electrical grid. The loss of such a plant could plunge the country into an energy crisis that could cripple its growing industry.

Once a developing country has an unsafe plant therefore these economic pressures could persuade it to continue dangerous operations. The Tarapur Atomic Power Station (TAPS) on the western coast of India which supplies power to the industrially important metropolis of Bombay can, for instance, be placed in such a category. TAPS was the first atomic power station to go in operation in the Third World. It was built on a turnkey basis by General Electric under an Indo-US agreement and commissioned in 1969. Defective fuel bundles supplied by General Electric led to widespread contamination of the plant and extremely high radioactivity levels. *Business India*, an Indian journal, recently reported in an extensive survey of India's nuclear programme: "TAPS is so heavily contaminated . . . that it is impossible for maintenance jobs to be performed without the maintenance personnel exceeding the fortnightly dose of 400 mrem in a matter of minutes. Thus the maintenance worker—who is often not an employee of TAPS—holding a spanner in one hand and a pencil dosimeter in the other, turning a nut two, three rotations and rushing out of the work area is a common phenomenon in TAPS." When asked why not shut down TAPS and decontaminate it thoroughly, a senior TAPS engineer replied: "Ideally, that should have been done in 1974 or earlier but there is such great pressure from the Department of Atomic Energy on us to produce power that we cannot shut down."

It must, however, be said to the credit of the Indian nuclear authorities, who control the best technical expertise in the nuclear field in the Third World, that they have taken considerable care to minimise the radioactivity problems in TAPS. India has a massive nuclear R&D programme which accounted for nearly one-fifth of the India government's R&D budget of Rs 3,200 million (about £220 million) in 1976-77. Despite this indigenous capability, Indian engineers had to seek the assistance of General Electric when fuel defects and related radioactivity problems first began to surface—and they obtained very little help. General Electric simply blamed them for mismanagement and lack of technical expertise. Even the US Atomic Energy Commission officials were then forced to prepare a note for Commissioner James T. Ramey in which they stated: "US manufacturers have extremely poor records of information disclosure to foreign

purchasers. Designs at times do not reflect all the regulatory items required in the US; at times design innovations are first tried by US manufacturers in overseas reactors. US manufacturers normally deal with foreign utility companies and short-cut their assistance to foreign governmental control groups (like nuclear regulatory agencies), which are sometimes in weak governmental positions." These discussions led Commissioner Ramey to emphasise that General Electric should be very careful with the nuclear plant it is setting up in Mexico. Mr Ramey pointed out to General Electric that "Mexico will have no technical back-up such as that which is available in India at the Bhabha Atomic Research Centre, and that GE should pay particular attention to assuring there is an adequate operations and maintenance staff, whether or not they have a contractual obligation to do so."

Dr Morris Rosen of the Nuclear Safety Section of the International Atomic Energy Agency has further listed several critical aspects of nuclear power plant exports to developing countries. At the time of the IAEA-sponsored International Conference on Nuclear Power held in Salzburg, Rosen pointed out that several power plants quite dissimilar to those that exist in supplier countries are being built in developing countries. For instance, he revealed that high seismicity at the site of two large reactors sold by West German vendors to Iran could result "in significant design changes in the nuclear facility; changes that influence the foundations, interface of structures, pipe requirements, supports and system components (including reactor internals). Thus, the eventual design of the Iranian facility as constructed may have some significant differences from modifications which will not have

undergone a detailed review by the regulatory bodies of the Federal Republic of Germany."

Rosen pointed out another example for which there was no reference plant—a similarly sized plant under construction in the exporting country which meets its safety requirements and therefore can be licensed. "The recent 2-loop reactor plant for Egypt, Korea, and the Philippines, is referenced to a 2-loop reactor plant under construction in Yugoslavia since 1974. This plant in turn had been referenced to an earlier 2-loop plant under construction in Brazil, which in turn had been referenced to a domestic plant in Puerto Rico. However, the review of the Puerto Rico plant was terminated in late 1972 . . . and it was determined not to continue with the project. If the Puerto Rico plant had been constructed, it would have undergone a systematic and detailed review by the US regulatory organisation and as a result of this review . . . a number of modifications would undoubtedly have been made. Thus, all of the previously mentioned exported 2-loop plants have undergone a rigorous regulatory review, and modifications that might have been required are not available for consideration."

The prime need of developing countries with nuclear programmes is strong and competent regulatory agencies—unfortunately something they very much lack. In the words of Mr Rosen: "At the present time, with little exception, the regulatory organisations of developing countries with active nuclear programmes can be classified as sub-minimal. In many cases they consist of less than 15 full-time staff members associated with nuclear power activities. This minimal staff may not be familiar with the disciplines of nuclear safety and may



South Korea: first steps into nuclear power, 1974.

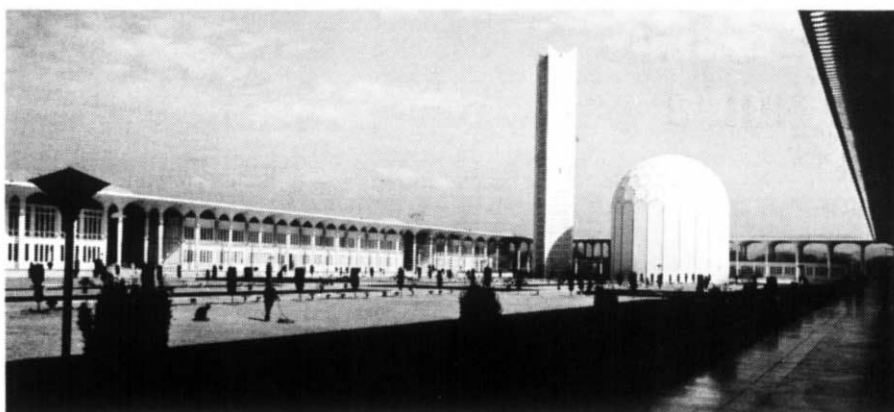
be in need of extensive training."

Without good regulatory agencies, developing countries can neither ensure they are being sold safe nuclear systems or that they are being operated safely. There are several obstacles to the setting up of a strong regulatory agency in a developing country. First of all, nuclear power is such a sensitive issue politically that few governments would risk setting up an organisation whose main responsibility would be to make critical reviews. Second, there is the question of cost which can be relatively very high for a small one- or two-reactor country. Third, there is the lack of technically qualified personnel.

To minimise their expenditures on nuclear programmes, most Third World governments hold the budgets of their regulatory agencies at very low levels both in foreign and local currencies. South Korea is a typical example. The Korean authorities have been making use of foreign consultants for their nuclear regulatory programme. Two separate studies have been completed, including one which gives detailed recommendations for the organisation of the regulatory body. But the South Korean government has still not put up the funds for implementing the recommendations. South Korea has also had an IAEA mission for evaluating site and safety reports. Though such procedures are useful, IAEA officials themselves do not consider them a substitute for a good domestic regulatory organisation. Like those of South Korea, regulatory units in Taiwan, Philippines and Mexico are also very weak.

At an IAEA-sponsored symposium on problems associated with the export of nuclear power plants, representatives of the Mexican Instituto Nacional de Energia Nuclear pointed out that the Mexican "regulatory body is still considered to be working under less than ideal conditions. For example, licensing actions have been taken with insufficient information and the construction schedule is such that in some cases INEN has had to make certain judgements without evaluating all the relevant safety considerations."

The Mexican case is particularly interesting as Mexico obtains its nuclear fuel through the IAEA and therefore agrees to comply with the applicable IAEA safety regulations. IAEA missions have visited the Laguna Verde Nuclear Project and identified several deficiencies. But little action has been taken. The paper quoted above points out: "Subsequent IAEA missions reported that little attention had been paid to previous recommendations. The Agency's Director General issued a second letter to Mexico's Ambassador to the United Nations. This letter was



Western technology meets eastern architecture: Pakistan's nuclear institute at Islamabad.

much more critical than the first one; however, the authors are not aware of the response to the letter nor of the actions taken by the Mexican government as a consequence."

The IAEA has a similar agreement with Yugoslavia but there the regulatory body is practically non-existent. A nuclear plant is under construction at Krsko with costs being shared by the republics of Croatia and Slovenia. Neither of them are interested in setting up a regulatory agency, nor are the federal authorities because of the considerable independence granted to the republics under the country's constitution. Perhaps the regulatory situation will improve when Yugoslavia becomes a two-reactor country. In India, there is considerable domestic competence, but the Atomic Energy Commission is both utility and regulator.

The IAEA's Director General, Dr Sigvard Eklund, invited a group of international experts to Vienna at the end of last month to discuss the safety issues thrown up by the Three Mile Island incident and the manner in which the agency should respond to them. The agency is currently preparing under its Nuclear Safety Standards (NUSS) programme, about 50 books in the form of Codes of Practice and Safety Guides. These will certainly help regulatory agencies in the developing countries to insist upon at least minimum requirements for safe operation of nuclear plants. But given weak regulatory bodies they will clearly have a limited impact.

An important activity that the agency could undertake is to send more safety missions to help IAEA member-states and possibly set up a system by which the agency could inspect power reactors. If the member-states of the agency were agreeable, there could be a resolution at the Board of Governors that they will adhere to the safety regulations stipulated by the agency or there could even be a specific international convention.

It is, however, doubtful whether

many Third World governments will react very favourably to such suggestions. They could be easily interpreted as yet another attempt by western governments to control the development of nuclear power in the Third World. A simple compromise like making IAEA recommendations only advisory and not mandatory could be a solution. But if countries lack the political will to ensure maximum safety or possess systems in which the utility is politically far more powerful than the regulatory agency, then regulatory work can turn out to be only a lot of paper work. IAEA safety missions could expect a considerable degree of hostility.

The inspection-related issues are likely to be discussed further at the meeting of the IAEA's Board of Governors later this month. The least that the agency could do is to step up the flow of information amongst member-states about nuclear mishaps which would help to spread understanding about how to cope with them. The Three Mile Island accident did show that proper training of operators is a matter of vital importance.

An activity that the IAEA is planning to undertake soon is the organisation of an emergency assistance system in the case of major nuclear mishaps. This would mean maintaining an international roster of experts on immediate call. The IAEA's nuclear fire-fighting brigade will, however, not be easy to operate. Experts will have to be flown from various parts of the world which will take a considerable amount of valuable time. Nonetheless, such a system will be a step in the right direction. In the TMI disaster, experts were required for several days.

In spite of international actions, the mishap at the Three Mile Island means that Third World governments with nuclear programmes will have to be more concerned with safety—and developed countries will have to ensure that safe technologies are transferred, especially to places where technical skills are still lacking. □

How Ghana cleaned up on soap but failed with nuts and bolts

In the last of his series, **Joseph Hanlon** looks at African attempts to turn 'appropriate technology' into small industries.

SCIENTISTS and technologists anxious to make a contribution to Third World development have in recent years often turned to 'appropriate technology', where there is a real need for science and engineering input. Several universities have set up AT centres, and one of the most successful—measured in terms of technology actually put into industrial production—is the Technology Consultancy Centre (TCC) at the University of Science and Technology in Kumasi, Ghana. But the TCC's experience shows that despite the need for scientific input, the key questions are not technical. Despite its success by the standards of other AT centres, the TCC is increasingly worried about its lack of impact in Ghana, and is implicitly raising questions about the whole concept of university AT centres.

Windmills always seem to be the first project that AT centres try: they seem such a nice, small, decentralised energy technology—just what the people 'out there' need. But there are no windmills at TCC. "People who have whole research programmes into windmills ask us why we don't have a windmill project. I ask where the application is—no one has ever come and asked us for a windmill," explains Dr John Powell, TCC director. The basis of TCC is that it "very seldom comes up with its own ideas. If you go to people and say 'You should do this', then when you go away nothing happens. But if they come to you, there is a faint chance it may happen."

This philosophy has led directly to the TCC successes. The first came just after the centre began in 1972. A local man, Mr Baffoe, who made laundry starch from cassava, came to the centre to ask about making paper glue. Schools traditionally make their own glue from cassava starch and the ash from plantain peel. (Plantain is a local banana-like food which is very common.) But the glue has a very short life and is not rewettable. Baffoe was passed on to Dr Rao in the university chemistry department, who found a non-toxic fungicide to use as a preservative, and chemicals for rewettability. Baffoe began making the 'spider glue' in the courtyard of his compound, and within a year was a fairly rich man.

The glue is an ideal example of appropriate technology. It is made almost entirely from local materials and replaces an imported product. Baffoe was able to supply most of Ghana's paper

glue needs, saving considerable foreign exchange, and his glue cost less than imported glue on the local market. Furthermore, it was small scale, used labour instead of capital, and it required some scientific input.

TCC's biggest success to date has been with soap making. Soon after the TCC opened, local small scale soap makers came to the TCC asking for help in improving the manufacturing methods. Engineering and chemical analysis produced an improved formula. With £10,000 from the Ghana government, work began on improved soap making tanks. The TCC committee, composed of two engineers, a pharmacist, and a chemist, "wanted a mini Lever Brothers," Powell said, but after much discussion, the committee was talked out of advanced technology and tanks using electric kettle elements were made. They "worked well with professional supervision on the campus. But it proved too sophisticated for rural soapmakers. I would expect that now, but didn't then—it was our first big project."

With help from the Intermediate Technology Development Group (ITDG) in London, G. Prakash, a consultant on small scale soap making at the Appropriate Technology Development Association in Lucknow, India, went to the TCC. He immediately redesigned the tanks to make them easier to control, and converted them for use on wood fires. Not only was it easier for the rural soapmakers to operate, it was a better technology—both capital and operating costs for the wood fired plants were less than half those of the electric plants. The project has been a considerable success, probably the biggest of any university AT project: more than 100 soap making tanks of TCC design have been made.

The soap is produced from palm oil and caustic soda. Within a few months of the first plant opening, however, caustic soda began to run short, and TCC turned to the chemical engineering department for help. There was an acetylene plant in Tema which imported calcium carbide and reacted it with water; the waste product, calcium hydroxide was simply dumped outside the gate. This could be reacted with sodium carbonate, which still has to be imported, but at half the price of the final caustic soda. So a simple electrically heated, mechanically stirred plant was built which produced caustic soda

for less than the market price. Six are now installed. Shortages of oil for soap making led to involvement in oil palm plantations, alternative oil seeds, and improved oil presses.

TCC is now a major operation, with 80 staff and a £100,000 per year budget. More than half that money comes from TCC's own production units, which manufacture soap, nuts and bolts, and equipment such as the soap making tanks.

Its biggest project to date is a pyrolytic converter to make charcoal, gas, and oil from the sawdust available in abundance from the sawmills around Kumasi. If wood is burned by the traditional charcoal burners, the charcoal produced amounts to only 10% of the original weight of the wood. By carefully controlling the burning temperature in the pyrolysis process, it is possible to produce 25% by weight of charcoal, plus 10% oil. No fuel input is required since the output gas is used to dry the sawdust, and the burning wood provides its own heat for pyrolysis. The Ghana Government, USAID, and the Georgia Institute of Technology have all helped in what is a quite sophisticated project—temperature must be carefully controlled, and there are special problems because the density of hardwood dust is different from that of softwoods used in this system elsewhere. Nevertheless, a pilot plant on the UST campus is already processing more than 1 tonne of sawdust a day and has reached 22% charcoal and 7% oil.

From the platform of these successes, Powell and others at TCC are now raising some questions. They point out that appropriate technologies can be surprisingly marginal. For example, there is a controlled price for soap, and when rocketing palm oil prices made it uneconomic to produce soap at the controlled price, the government gave a subsidy to Unilever, but not to small soap makers. Eventually, TCC and other soap makers had simply to ignore the controlled price. Similarly, several TCC projects depend on small imported items such as bearings and colourings that are simply smuggled in because import licences are not available.

Animal feed production from brewers spent grain is a case in point. Grain left over from brewing ferments within a day, and Kumasi Brewery throws away 100 tonnes a week. The Department of Biochemistry at UST analysed the grain for a local entrepreneur and found it suitable as an animal feed (a common use of spent grain in other countries). TCC developed an effective drying process, involving a press and sun-drying, and the entrepreneur was soon in business. The nearby Guinness Brewery bought a highly mechanised drying plant at about the same time.

This runs at a loss, despite charging 6 cedis a bag for dried spent grain, while the local entrepreneur with his simple technology makes a profit at 4 cedis a bag. But he is surviving in business only because the Kumasi Brewery has consistently been refused import licenses for machinery like that used by Guinness. But how long will that continue?

The successes of TCC, however, only make clear its biggest failure. "Contrary to expectation, entrepreneurs have been very slow to follow the lead given by the university," comments vice chancellor E. Bamfo Kwakye, a strong backer of TCC. He thought that entrepreneurs would take things out of their hands, as with soap and glue, but this has not happened. Many products, such as nuts and bolts, are still manufactured on campus. There seem to be three reasons—the location of TCC, the nature of Ghanaian entrepreneurs, and economic factors.

Many small businessmen are unwilling to come to the large university campus outside Kumasi. So TCC has decided to go to them. It proposes two Intermediate Technology Transfer Units in the middle of existing informal industrial areas. These would actually manufacture products, but they would also provide training, designs, and other help to upgrade existing craftsmen. One unit will be in Suame Magazine, Kumasi, Ghana's largest informal industrial area, which has thousands of craftsmen, like fitters and carpenters. The unit there will be involved in blacksmithing, carpentry, and so on, and will have its own machine shop. The second unit will be in Tamale, in the north, and concentrate on the manufacture and repair of agricultural equipment. Funding will come from the US and Canada.

The second problem is a lack of Ghanaian industrial entrepreneurs. Ghana is well known as a trading country. It has large markets, and every family seems to have one member involved in trading or transport. This may be part of the colonial heritage—the British saw Ghana as a source of raw materials and a market for manufactured products, but they were rarely directly involved in the internal economy, as in their other African colonies. Local people were encouraged to trade, but not to manufacture goods which might compete with Britain. Trading requires different skills from manufacture, resulting in a dearth of industrial management expertise in Ghana, and this is one factor cited as a reason to opt for advanced technology, which requires fewer managers than decentralised AT. In any case, Ghanaians find trading much more pleasant—trips and chats are part of the work, the hours are flexible, and



TCC's pyrolytic converter: sawdust is burned in the tank and the gases condensed in a water collar to produce oil. Left is TCC director Dr John Powell.

profits can be very high. Industrial work, on the other hand, is physically hard, much steadier, and often noisy and dirty.

The third problem is economic. As Sally Holtermann, an economist who recently studied TCC for the Intermediate Technology Development Group in London, points out: "in an economy where the activity is to be carried out by private enterprise, entrepreneurs are not going to invest in plant and equipment that is not going to yield them a profit." Sometimes Powell recognises this. But he also told me: "You can get awfully confused with economics. Sometimes I think it is better to avoid economics and concentrate on technology."

The result is best shown by nut and bolt making. Because of import restrictions, craftsmen in Suame Magazine were having trouble getting coach bolts for the wooden bodies they make for passenger lorries. So the TCC imported some used equipment, including capstan lathes, and set up a shop on campus making nuts and bolts. It seemed an obvious technology to transfer—but it hasn't been, for reasons now becoming clear. The market is dependent on import restrictions, since imported nuts and bolts, when they are available, are one-third the price of those made at TCC. Second, making one-off motor spares and machine parts is just as profitable for the entrepreneur with this machinery, and much more interesting than long production runs of bolts. And third, TCC found out only last year that for four years its nut and bolt shop has been losing money. It now makes a profit, but not enough to interest a Ghanaian businessman.

The response of TCC has been to question the attitude of the Ghanaians. "If a nation is ripe for development, it will go forward, no matter what the development technology. India and China have reached this psychological take-off point, but it hasn't happened

here," Powell argues. "The average Ghanaian trader, if he can't make his profit today, isn't interested. Few people here realise that development does not take place overnight—you have to work for your children's children." The answer is not that simple, though. Could it be that Ghanaians are suggesting in practice that although a technology meets academic definitions of 'appropriateness,' it may not be right for Ghana?

TCC has received strong official backing. It was even mentioned in the government's last five year plan. But it has had little contact with government research and development units. It gets strong backing from the university, and practical support from a few people like Kwakye. But there is virtually no participation from other Ghanaians at the university. Nearly all of its work is done by expatriates at the university, or by Ghanaians actually on the TCC staff, some of whom are extremely dedicated. Even Powell mentions the almost complete lack of student interest in TCC. Nor have TCC's successes increased involvement; in fact, the opposite has occurred. After the success with spider glue, many faculty members refused to cooperate because they felt they were being exploited. And at the centre itself, it is the strength of expatriate Powell that keeps TCC running. People may come in at 7.30 each morning when he is there, but they don't when he is on leave.

Powell himself may have the answer. "AT will only work when it is done as in India—by people for themselves. Here, it's not Ghanaian professors working for Ghanaian peasants. The whole thing is an expatriate activity, an oboroni [white man's] hobby. I don't have to use the soap made in my factories. It's technology for them, not for us." But when the other alternative is Unilever, perhaps all Ghana can do is choose between different oboroni technologies. □

news and views

Fibronectin: a function at the junction

from Clive Lloyd

THE fact that suspended tissue cells attach and flatten on suitable surfaces is not only experimentally convenient but a powerful example of the trans-action between internal musculature and the external environment. Involvement of the cell's actomyosin system in making contact and generating movement began to emerge some time ago and now there is evidence to promote the involvement of the extracellular molecule, fibronectin (also known as LETS protein), in forming organised cell adhesions. The significance of these recent findings is that fibronectin and associated extracellular proteins—perhaps in a process common to many biological events—stimulate the formation of cytoplasmic organisation. This raises questions about the nature of the transmembrane steering linkage between the two systems.

At the observational level tissue culture cells which are spread on a culture dish become spheroidal when brought into suspension but will flatten once more when restored to some suitable surface. At the molecular level, this cycle of events can be described in terms of the development of the internal musculature by which a cell grips the substratum (Rees *et al.* *Nature* **267**, 124; 1977) and then the loosening of this grip (and hence the loss of asymmetrical cell shape) in the presence of dissociating agents. Components of this actomyosin system can exist in different states of organisational complexity (see Lazarides and Revel, *Sci. Am.* May 1979, for a graphic account) but conspicuous microfilament bundles which characterise and maintain the well-spread cell often seem to 'crystallise' out from localised points of cell-cell or cell-substratum contact. Because not all surfaces support active cell spreading, the nature of the congenial substratum is clearly important in setting up microfilament bundles and this is where fibronectin seems to play a major part. For instance, transformed cells deficient in microfilament bundles,

spreading ability and cell surface fibronectin (LETS) can be converted back to a more normal spread morphology with abundant microfilament bundles by the administration of fibronectin (Willingham *et al.* *Cell* **10**, 375; 1977; Ali *et al.* *Cell* **11**, 115; 1977). Conversely, the dissociating agent trypsin, at low levels, removes LETS from the cell surface (Hynes *Proc. natn. Acad. Sci. U.S.A.* **70**, 3170; 1973), perturbs the organisation of cytoplasmic microfilament bundles and converts well-spread cells into rounded ones (Pollack and Rifkin, *Cell* **6**, 495; 1975). The distribution of fibronectin is consistent with its role in cell attachment for it can occur as a fibrous meshwork sandwiched between the cell and the substratum and is also observed between cells in contact (see Yamada and Olden *Nature* **275**, 179; 1978 for general review).

These general properties are an already accepted chapter of the fibronectin/LETS saga but a more detailed examination of the correspondence between this extracellular molecule and the cytoskeleton will be required before any reasonable molecular explanation of their relationship emerges. Towards such an explanation, Hynes and Destree (*Cell* **15**, 875; 1978) used double-label immunofluorescence to tackle the problem. They show that actin microfilament bundles close to the cell's substratum are often (but not always) co-extensive with fibronectin outside the cell because the rhodamine anti-fibronectin and fluorescein anti-actin image are, to a reasonable degree, superimposable. The resolution of this technique is suggested to be not greater than 200 nm, but still more recent evidence suggests that any transmembrane gap between these parallel systems of fibres (if one exists at all) is of the order of 8–22 nm. I. I. Singer (*Cell* **16**, 675; 1979)

reported electron microscopy of thin sections cut through regions where internal microfilament bundles and external fibronectin-containing fibres meet, proposing that the electron-dense plaque where the two systems come together be called the fibronexus. Sections cut approximately parallel to the plasma membrane gave the appearance of microfilament bundles overshooting the plasma membrane and continuing on through the extracellular space—a conclusion that Perdue (*J. Cell Biol* **58**, 265; 1973) had previously reached by an electron-histochemical study of chick fibroblasts. With the benefit of ferritin-conjugated antibodies to fibronectin (probably not available to Perdue at that time) Singer shows that the extracellular portion of this transcellular highway contains fibronectin. This agrees with Hynes and Destree's observations that "actin microfilament bundles frequently terminated before the periphery of the cell, whereas the co-linear fibronectin often continued further towards the edge of the cell and even beyond it". Singer tilted his sections through 40° in the electron microscope to see if there was any hidden gap between the two systems but, given the limits to resolution, he saw none and the structures remained co-linear throughout. This tends to suggest that where fibronectin and microfilament bundles are related, they are related in a 1:1 manner, but whether this relationship is co-axial or of overlapping ends with some special binding structure in between, is an open question. The fibronexus, then, would seem to be synonymous with cell adhesion plaques except that a role for fibronectin in forming such junctions is now more clearly claimed.

The recent paper of Thom *et al.* (*J. Cell Sci.* **35**, 281; 1979) bears directly on this and concludes that fibronectin is necessary for the formation of organised junctions, but not entirely sufficient. Interference reflection microscopy is a technique that

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produces a contour-map of the under-surface of the cell—the focal points of close contact with the glass ('feet') being darker than more distant areas of the cell body. These 'feet' are known to be the terminations of micro-filament bundles (Heath & Dunn *J. Cell Sci.* **29**, 197; 1978) and in the electron microscope they have a structure resembling half of an intercellular junction of the adherens type (see Heaysman, in *Locomotion of Tissue Cells*, Ciba Symp. No. 14, 187; 1973). Thom *et al.* demonstrate that a line of rat fibroblasts attaches and spreads on glass pre-coated with fibronectin isolated from chick serum but that the interference reflection image of 'feet' remains poor and not fully organised unless other components of serum are also present.

Such other components may therefore either directly participate in the organised adhesion or may merely maintain the cell until it can tailor its own fibronectin into more favourable arrangements than occur adsorbed onto pre-coated glass.

As for the internal face of the junction, Singer echoes the view that the fibronexus may be the nucleation centre for actin bundle formation, quoting Lazarides and Burridge's observation (*Cell* **6**, 289; 1975) that the Z-band protein of skeletal muscle (α -actinin, which holds actin thin filaments in ordered arrays) is also present at the termini of microfilament bundles in non-muscle cells. In keeping

with this are Geiger and S. J. Singer's findings (*Cell* **16**, 213; 1979) that various specific extracellular ligands cause receptors to accumulate into patches and caps on the surfaces of various cells at the same time that α -actinin forms sub-caps at the cytoplasmic face of the membrane. Inferences to be drawn from this include the possibility that α -actinin provides the hinge between actin and integral membrane receptors, or that α -actinin may collect together actin-linked receptors. This takes on further shape for adherent fibroblasts in Badley *et al.*'s recent work (*Expl Cell Res.* **117**, 231; 1978) in which cell bodies were removed from glass by a stream of buffer, leaving 'feet' behind in the same pattern as they existed in the intact cell. All of the components discussed so far in terms of a trans-membrane assembly could be detected by immunofluorescence at these adhesion plaques: actin, myosin, tropomyosin, α -actinin, LETS (but not tubulin or serum albumin).

Putting all this together, fibronectin is a sticky protein which agglutinates red blood cells (Yamada *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3158; 1975); has a binding site for collagen (Hahn and Yamada, *Proc. natn. Acad. Sci. U.S.A.* **76**, 1160, 1979); may be closely associated with sulphated proteoglycans (Perkins *et al. Cell* **16**, 941; 1979) and may therefore help organise biological matrices. It is concentrated at points of cell contact and can be traced

across the membrane to actin micro-filament. Just as the external contact may be stabilised by components of the extracellular matrix, so the organisation at the internal face of the junction may be underpinned by α -actinin. However, any communication across the membrane is likely to be two-way because external fibronectin stimulates internal microfilament bundle formation while, conversely, breakdown of the bundles by cytochalasin B (Kurkinen *et al. Expl Cell Res.* **111**, 127; 1978) causes the release of fibronectin from the cell surface.

Of course, there is strong evidence that the cytoskeleton can be modulated indirectly by the addition of cyclic nucleotides to cells (see Pastan and Willingham *Nature* **274**, 645; 1978) but in cases where the contractile cell flexes its muscle against the outside world (in attaching to cells and other biological surfaces, during cell migration and phagocytosis), a direct linkage is likely to be of prime importance. It has been suggested that many classes of external peripheral proteins cluster against one common class of trans-membrane protein which alone holds the hot-line to cytoplasmic microfilaments (Bourguignon and Singer, *S. J. Proc. natn. Acad. Sci. U.S.A.* **74**, 5031; 1977). The name given to this postulated molecule is perhaps an eloquent summary on the current debate about how outside and inside hinge together—it has been called 'protein X'. □

Nucleic acid statics and dynamics

from Stephen Neidle

RECENT years have seen a revival of interest in structural studies of both nucleic acids themselves and of model systems for them. The impetus for this can be attributed to various causes—it comes from work on chromatin and transfer RNA; from visualisation of the structures of nucleic acid fragments, sometimes complexed with drug and mutagen molecules, and from the emergence of new and powerful techniques, especially the advent of high-resolution nuclear magnetic resonance spectroscopy. It was perhaps timely that at a recent meeting on 'molecular stereodynamics'* half the sessions were concerned with such topics.

Even though hypothetical non-double-helical models for DNA are largely discounted (Arnott *Nature*

278, 780; 1979; Crick *et al. J. molec. Biol.* **129**, 449; 1979), there is lively interest in non-classical double helices. Levitt's analysis from empirical energy function calculations has suggested that in solution DNA has about 10.5 rather than 10 base pairs per turn, with the base normals markedly tilted from the helix axis and the base pairs themselves having propeller-like twists (*Proc. natn. Acad. Sci. U.S.A.* **75**, 640; 1978). D. M. Crothers (Yale) presented data from transient electric dichroism studies on drug-DNA intercalation complexes which seem to support some features of the Levitt model. In these structures, the drug chromophores are tilted by an average of about 20° from the perpendicular to the helix axis; nonetheless the nucleic acid was not observed to bend or kink *à la* Sobell (*J. molec. Biol.* **114**, 333; 1977). Such observations can only be rationalised by evoking partial unstacking and tilting of base pairs at the intercalation site. This in turn suggests a base-pair twist angle in DNA of 17°,

as does Levitt's model. L. S. Lerman (State University of New York, Albany), the originator of the intercalation concept, reported on his studies of DNA torsional elasticity probed with spin-labelled intercalators. Evidence was presented for substantial internal movement within the double helix; a single base pair oscillates about the helix axis with a root mean square amplitude of about 5°.

Nevertheless detailed structural data on nucleic acid-drug intercalation complexes (as opposed to hypothetical models), is still largely lacking. A favoured indirect approach to this problem is through the study of model systems. The pioneering crystallographic studies of H. M. Sobell (University of Rochester) on drug-ribodinucleoside complexes have clearly illustrated the intercalation phenomenon at this level. Sobell interprets these structures in terms of DNA complexes possessing kinks at the intercalation sites—this in turn has led him to suggest that DNA is a

*A conversation on Stereodynamics of Molecular Systems was held at the State University of New York, Albany, on 23–24 April, and was organised by R. H. Sarma under the sponsorship of the State University of New York and the General Electric Company.

dynamically kinked-unkinked structure. The basis for kinking, alternating sugar pucker of residues at the intercalation site, was challenged by the results of H. M. Berman (Institute for Cancer Research, Philadelphia) and S. Neidle (King's College London), who find that for dinucleoside complexes, the nature of the sugar pucker (a parameter known to be 'soft') is probably unimportant. A. Rich (Massachusetts Institute of Technology) has also examined the crystal structures of several of these model intercalation complexes, and concludes that the expression of sugar flexibility in them depends on whether or not the particular drug involved interacts with the phosphate group in the backbone. It is not clear to what extent such drug-ribodinucleoside complexes are meaningful models for the drug-DNA complexes themselves. As Berman emphasised, great caution is needed in extrapolating from the model systems. Indeed, the data presented by Crothers suggest that the model complexes may be related to the DNA ones in rather more subtle ways than hitherto believed.

High-resolution nuclear magnetic resonance (NMR) is in principle a powerful probe of oligonucleotide conformation in solution, so as to provide data complementary to that from the crystal structures, as well as dynamic information. However, complete analyses of the spectra in terms of molecular geometry of even a relatively simple system such as a drug: dinucleoside one, have not been made. In spite of this proviso, NMR has been most useful in the study of many aspects of intercalation complexes. T. R. Krugh (University of Rochester) has shown that the simple intercalators such as ethidium show a marked sequence preference on binding, with pyrimidine-purine ones being preferred. This is the reverse of the preference shown by actinomycin D, which Krugh and his associates have shown to exhibit sometimes unexpected nucleic acid-binding behaviour. For example, although it has been known for many years that the drug requires a guanine at its binding site (and thus does not bind to poly(dA-dT).poly(dA-dT)), the anti-tumour intercalator daunomycin, which does bind to this polymer, actually creates noncompetitive actinomycin binding sites.

D. J. Patel (Bell Laboratories) has been examining by proton NMR the structural dynamics of synthetic polynucleotides both with and without intercalating drug. He presented further evidence for the 'softness' of

the sugar pucker in such systems. However, it is not clear whether, at least for an intercalative complex, the mixture of sugar conformers observed indeed represents a true alternating sugar situation (as propounded by Sobell), or a mixture of conformational populations. The power of the NMR method in conjunction with molecular model-building was well shown by R. H. Sarma (State University of New York, Albany) and his colleagues, who have examined models for the novel vertically stacked double helix suggested by Olson (*Proc. natn. Acad. Sci. U.S.A.* **74**, 1775; 1977). The concept of nucleic acid flexibility was further taken up by N. R. Kallenbach (University of Pennsylvania), who has examined the equilibrium and dynamics of transient base-pair breakage by following proton exchange rates, a simple yet powerful structural probe. Kallenbach concludes that indeed there are transient open states (analogous to Sobell's picture of DNA 'breathing').

Gleaning of dynamic properties from crystallographic results is necessarily an indirect process. However, both Rich and S.-H. Kim (University of California, Berkeley) valiantly attempted to confront this problem armed with their data from both small oligonucleotide and transfer RNA studies. Nucleic acids are inherently flexible, and tend to respond to their environments (be they drugs, counter-ions or proteins), in well defined, or at least preferred ways. It is apparent that even short stretches of double helix can easily be bent under the influence of directional ionic forces; in transfer RNA the axis of one helical stem has been observed to bend by some 25°. More controversially, Rich suggests that DNA winding around the histone core in chromatin is due to histone location on only one side of the double helix. The related problems of solvent accessibility and exposed surface area in nucleic acids were discussed by Kim, who emphasised the importance of knowing which are in fact the penetrable regions of these molecules, when considering possible interactions. By considering the Van der Waals radii of the atoms in a nucleic acid, he has shown, for example, that in (classical) B-DNA the minor groove can accommodate only very small probes. This distinctive method of looking at nucleic acids gives essentially a static picture; however Kim considers that the use of individual atomic thermal parameters as a description of motion may well lead to knowledge of dynamically accessible surface areas.

There are perhaps more distinct theoretical approaches to the prediction of nucleic acid conformation



A hundred years ago

THE Italian State Secretary for Public Buildings has sanctioned the plans submitted to him for the construction of an observatory on the summit of Mount Etna.

THE Anthropological Exhibition at Moscow seems to be one of great interest. It is contained in a vast building lent by the Minister of War, and is used in winter for drilling soldiers, and the exhibition has been rendered as picturesque as it is scientific. A garden which has been arranged most artistically for the purpose presents, among other features, a very remarkable "palaeontological valley." This is planted with lycopods, gigantic ferns, and other fossil plants; this forest is inhabited by models representing megatheriums, mammoths, ichthyosaurs, &c. On miniature mountains, the age of which is indicated by artificial geological sections, are shown *fac-similes* of Russian, French, Danish, and other tumuli. Besides this, an ethnological garden is peopled with models representing the principal human types, especially those of Russia. There is, besides, a remarkable anatomical and craniological exhibition. Altogether this is one of the most remarkable anthropological exhibitions ever brought together, and has been an immense success.

DR MICLUCHO MACLAY the Russian explorer, with an Italian, Chevalier Bruno, and Capt. Leeman, have sailed from Sydney for New Guinea, in the American schooner *Laddie*, F. Caller, chartered for a twelvemonth's cruise. 2,500*l.* has been spent on the equipment. The expedition is intended to be both scientific and commercial. New Caledonia, New Britain, and other islands are to be visited.

From *Nature* 20, 5 June, 131, 134; 1879.

than there are actual practitioners of such arts. The experimentalist tends to treat all of them with a healthy scepticism. It was thus gratifying that S. Broyde (New York University), reporting on her semi-empirical energy calculations with B. Hingerty (Oak Ridge National Laboratory), with a model for the coil form of the poly(U) conformation, was able to show that the low-energy form suggested agrees with hydrodynamic and NMR data on poly(U). W. K. Olson (Rutgers Univer-

sity) has used the methods of polymer chain statistics in assessments of DNA flexibility dependence on chain length. Her results suggest that very small changes in just a few conformational parameters of individual residues lead naturally to very considerable flexibility in the polymer as a whole.

These contributions signify some of the more promising directions in which nucleic acid structural studies are going. All of these, however 'biological' the nucleic acid environment may be in an experimental situation can only provide *in vitro* data. One must look in the future to techniques such as those involving NMR described by S. S. Danyluk (Argonne National Laboratory), for information from intact biological systems. □

Scrambling of Rydberg states by thermal radiation

from Peter Knight

ALL atomic systems are immersed in a bath of blackbody radiation, a background thermal field characterised by the ambient temperature of the body. For the most part physicists interested in radiative transitions of excited atoms just ignore the existence of this field, principally because the number of blackbody photons with a frequency close to the atomic transition under study is vanishingly small, so that stimulated processes due to background thermal fields are negligible. Nevertheless, if the transition frequency is small enough, the Planck distribution of thermal photon numbers ensures that stimulated emission and absorption will begin to take over from purely spontaneous radiative decay.

Recent experiments by Gallagher and co-workers at the Stanford Research Institute have indicated that transitions between very highly excited Rydberg states are sensitive to background thermal fields. At the transition frequencies that interest them, the thermal photon occupation number at room temperature can be as high as 10. The stimulated absorption and emission of this background radiation rapidly redistributes an initially excited state population among its neighbours and considerably shortens the lifetime of some states. Consequently it would seem quite difficult to observe the decay of a

single isolated Rydberg state in these circumstances: after a very short time a distribution of states rather than a single state exists.

In their first paper, Gallagher and Cooke (*Phys. Rev. Lett.* **42**, 835; 1979) report the observation of the shortening of the sodium radiative *np* lifetimes by a factor of 3 by 300 K blackbody radiation induced emission and absorption. They measured the effective lifetime of the 17p and 18p states of sodium produced by sequential excitation by two dye laser pulses driving the $3s \rightarrow 3p \rightarrow 18p$ or 17p transitions. These states can be detected by field ionisation by a d.c. field, with a pulsed technique which allows them to monitor the time-evolution of the excited state population. Their results indicate that the lifetimes are a factor of three shorter than the calculated $T=0$ K lifetimes, in agreement with their fairly simple analysis. They believe discrepancies between theory and experiment for transitions observed by other workers can be explained by thermal background effects. They also calculate that radiative redistribution of population among neighbouring states of different *l*-quantum number due to the thermal field can be as important as collisional redistribution. This could well have astrophysical implications. Gallagher and Cooke also calculate the a.c. Stark frequency shifts produced by the thermal field. These had, in fact, been investigated theoretically some years ago by Barton (*Phys. Rev.* **A5**, 468; 1972) and by Knight (*J. Phys.* **A5**, 417; 1972), and the results of Gallagher and Cooke are in agreement with those earlier quantum electrodynamic calculations. The thermal field shifts all the close-lying Rydberg states upwards in energy by an equal amount of the order of kilohertz. Low-lying states are shifted by a much smaller amount according to the theoretical work of Barton and Knight. So photoabsorption from a low-lying state to a Rydberg state may well exhibit temperature-dependent shifts, although these have yet to be seen. In a further paper, Gallagher and Cooke discuss the use of Rydberg atoms as a detector of 300 K blackbody radiation (*Appl. Phys. Lett.* **34**, 369; 1979), hinging on the sensitivity of field-ionisation as a monitor of highly excited state populations. They suggest that temperatures of less than 25 K could be detected by Rydberg-atom systems.

It is interesting to the theorist that the atom immersed in a radiation field is quite different from an isolated atom. The radiation bathing the atom also dresses it, so that what started as an isolated state is scrambled into a distribution of nearby states with transition frequencies determined at least in

part by the local environment. Hot atoms aren't just moving faster than cold atoms (and therefore the ensemble emitting a wider Doppler-broadened line): each hot atom is distinctly different from a cold atom in its radiative properties. □

Proteins of iron metabolism

from Pauline Harrison and E. R. Huehns

THE main topics discussed at the recent, fourth International Conference on Proteins of Iron Metabolism*—transferrin, the iron transport protein, and ferritin, the iron storage protein—are both in their own way still subject to considerable controversy. Transferrin has long been known to have two iron-binding sites, but whether these are identical structurally and, if different whether this has physiological importance, as Fletcher and Huehns proposed (*Nature* **218**, 1211; 1968), has been argued about at the previous three meetings.

B. Gorinsky (Birkbeck College, London) presented a '6Å resolution model' of rabbit transferrin while J. E. Abola from Pittsburgh had studied hen ovoid-transferrin. The rabbit molecule consists of two lobes which can be described approximately by ellipsoids. The longest axes of these ellipsoids are inclined at about 30° to one another. The maximum overall dimensions of the molecule are approximately 100 × 50 × 40 Å. Both lobes exhibit clefts in the vicinity of the join. In one lobe the cleft is close to a column of strong electron density which could be a helix, and on the opposite side of this lobe there is a strong feature which at higher resolution may prove to be a β sheet. From other work it can be concluded that there is one iron-binding site on each domain and that the N-terminal binding site can be distinguished from the C-terminal one. Several investigators (J. Williams, Bristol; P. Aisen, New York; H. G. Van Eijk, Rotterdam) utilised electrophoresis in 6 M urea, as described by Seal and Mackay (*Biochim. biophys. Acta* **453**, 250; 1976), to separate the four species of transferrin—apotransferrin, diferric transferrin, and the two species of monoferric transferrin, one with iron on the N-terminal site only and the other with iron on the C-terminal site only—and to measure their distribution after the addition of iron to apotransferrin *in vitro* and in

*Held in Davos on 17–21 April 1979.

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plasma. The results show that the distribution of iron on the two binding sites is not random both in *in vitro* loading experiments and in plasma *in vivo*. How far the results will confirm the detailed predictions of the so-called Fletcher-Huehns hypothesis has not been determined. But the non-equivalence of the two iron-binding sites and the non-random distribution of iron on them in plasma seems to be established and is presumably of some physiological importance.

An interesting approach to the evolutionary significance of transferrin was that of F. M. van Bockxmeer and E. H. Morgan (Western Australia) who compared the binding of transferrin to heterologous receptor cells and, from the relative affinities, constructed an evolutionary family tree for transferrin. Several investigators attempted to isolate the transferrin receptors from reticulocytes (J. Glass, Boston) or the placenta (E. B. Brown, Hong Kong and St Louis), but this work is still in its early stages.

Since the conference in 1977, advances have been made in the determination of the structure of the iron-storage protein, ferritin. P. Harrison (University of Sheffield, UK) described the interpretation of the 2.8 Å resolution electron density map of horse spleen apoferritin, in terms of a symmetrical arrangement of 24 equal subunits of 60% α -helix content. The sequence determination is nearly complete (R. R. Crichton, Louvain-la-Neuve, Belgium) and interpretation of the three-dimensional map with sequence data is in progress. A complete description of this structure, now in sight, opens up the prospect of being able to settle arguments on how the ferritin molecule accumulates and releases its iron. Perhaps it may also lead to eventual agreement on the problem of ferritin heterogeneity, still a confused area. Differences in ferritin molecules both within and between preparations have been observed by electrophoretic and immunological methods. It now seems clear that these differences cannot all be explained by a single unifying hypothesis, such as that of J. Drysdale (Boston) who has proposed that all ferritins are heteropolymers of two subunits of different primary structure. Observations of apparent subunit size differences persist in some ferritins, although some types of reported heterogeneity may be methodological artefact. It now seems

unequivocal that ferritins from different cell types may differ in charge. For example, this was reported for ferritins from rat liver Kupffer cells and hepatocytes by J. W. Halliday (Brisbane, Australia) and from bullfrog red cells and liver cells by E. C. Theil (Raleigh, N. Carolina) who also reported amino acid composition differences.

Several workers produced evidence that iron-loading changes the 'isoferritin' pattern, but it is not clear whether this results from selective synthesis and/or degradation, or from post-translational modification. M. Worwood (Cardiff, UK) found that a fraction of serum ferritin is modified by glycosylation, a fraction which may alter in diseased states. These workers also reported that the immunological properties of circulating ferritin in patients with malignant disease are similar to those of ferritin from normal spleen. A molecule with high iron-content (significantly different from ferritin) has been found in *E. coli* grown on iron-rich media by E. R. Bauminger (Jerusalem and Rehovoth, Israel), suggesting that a means of conserving iron or of compartmentalising unneeded iron, is more widely required than hitherto recognised. On the other hand, iron-storage may not be the whole story with ferritin. Immunosuppression in patients with Hodgkin's disease may be partly linked with the presence of ferritin on their lymphocyte surfaces.

The last session of the conference concerned the management of transfusional iron overload, and although there were no new approaches, several investigators confirmed that maximal iron excretion could be best achieved using daily subcutaneous desferrioxamine with vitamin C supplements. The production of animal models of iron overload was also described by G. McLaren (Cleveland) and M. Arai (Okayama, Japan). □

Ion-driven inertial fusion

from Richard C. Arnold

THE first experiments using pulses of light ions to implode a hollow target, which might in the future be scaled up to produce inertial-confinement fusion, have been reported (*Phys. Rev. Lett.* **42**, 610; 1979) by a group at the

Sandia Laboratories in the US.

Previous experiments have been carried out for some years with lasers and electron beams (see Stickley *Phys. Today* **50**; April 1978); thermonuclear burn has been achieved in both cases. The light ion driver experiments reported, with pulse energies of 0.5 terawatt, are as yet at least one order of magnitude too weak to produce a measurable neutron yield (in fact, no attempt was made to use deuterium or tritium), but are sufficiently powerful to allow meaningful diagnostics of target response.

Ion beams can be produced with high electrical efficiency, and deposit their energy very effectively in the metallic surface of a fusion target. In these respects light or heavy ion-beam drivers are superior in principle (compare *Sci. Am.* **239**, 50; 1978) to existing laser systems. However, the physics and technology required to arrange focusing of intense ion beams on a small target for implosion purposes has been questioned, particularly for light ions of low kinetic energy (~ 1 MV) and high currents (≥ 1 MA) required for an inertial-fusion reactor. The Sandia experiments have demonstrated that a major fraction of their ions can be deposited on thin (~ 4 μ m) conical target shells about 1 cm in diameter, driving the shell inward in an axially symmetrical implosion. A plasma temperature of 12 eV was achieved at the peak of collapse (thermonuclear burn in this configuration would probably require about two orders of magnitude greater temperature).

The pulsed-power technology used in these light-ion experiments is relatively inexpensive compared with the heavy-ion multi-gigavolt accelerator technology (see *Nature* **276**, 19; 1978) now under development with a view to commercial power applications. As a consequence, fusion target experiments with light ions will probably be done much earlier than in the US heavy-ion programme. The latter technology, however, has many potential advantages for commercial reactor design. For example, heavy-ion beam propagation over several meters in a reactor vessel is expected to be classical and relatively straightforward; while for light ions, such long-distance propagation must be carefully arranged through a pre-ionised neutralising plasma channel, raising serious questions of possible propagation instabilities at the low kinetic energies used. Propagation and focusing on a small spot ($\ll 1$ cm) thus need to be demonstrated with light ions, and several groups in the US (at Sandia, Cornell University and the Naval Research Laboratory) are investigating this question. □

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A decade of plate tectonics

from A. Hallam

J. TUZO WILSON is widely acknowledged as one of the founding fathers of the new global tectonics. Because he has been successively Professor of Geophysics and Principal of Erindale College in the University of Toronto, before taking up his present position of Director of the Ontario Science Centre, it is appropriate that the university recently held a conference* to honour his multifarious contributions to earth science. Although various topics were discussed, including the early thermal history of the Earth, electromagnetic studies of the crust and the origin of ore deposits, the dominant theme was plate tectonics. Approximately a decade has passed since plate tectonics became generally accepted, and the meeting provided a valuable means of finding out how the subject was now being studied in a range of disciplines and of taking stock of the current state of knowledge.

Much of the critical research leading to the earth sciences revolution was in the fields of rock magnetism and seismology, and several leading workers were present to outline recent advances. Both A. Cox (Stanford University) and E. Irving (Earth Physics Branch, Energy, Mines and Resources, Ottawa) indicated how detailed palaeomagnetic studies in alliance with geological research demonstrate that extensive sectors of the Western Cordillera of North America had either rotated or been translated northward for substantial distances in the late Mesozoic and early Tertiary. While Irving was concerned essentially with northward movement of two landmasses, Wrangellia and Stikina, Cox dealt mainly with inferred rotations. To apply sailing terms, the structural geologist studies pitch and role but only the palaeomagnetist can determine the yaw. D. York (University of Toronto) showed how a combination of palaeomagnetic and isotope studies could help to unravel complex events in the Precambrian and confirm, for example, the reality of the polar wandering event about 10^9 years ago known as the Grenville Loop.

A quite different approach to rock magnetism was adopted by F. J. Vine (University of East Anglia) who concerned himself with the record of reversals of the geomagnetic field revealed by ocean floor anomalies, and demonstrated how further detailed re-

search could reveal information on variations in intensity of the field in space and time, which can delimit changes in the strength of the dipole and non-dipole field components with time.

Seismological data originally provided strong evidence for the delimitation and character of plate boundaries, and attention is now being directed more to seismicity within plates. L. R. Sykes (Lamont-Doherty Geological Observatory, New York) indicated that intraplate earthquakes tend to be concentrated along pre-existing zones of weakness affected by the youngest orogeny that predates the opening of the present oceans. Many such zones, marked by fault and suture lines and failed rifts, were reactivated during the early stages of continental separation. In contrast, intraplate shocks rarely occur within the older oceanic lithosphere or within ancient continental cratons.

The seismic reflection profiling method, developed by the oil industry for the study of sedimentary basins, is now being applied to the study of the structure of the continental basement. The most interesting result so far, as described by J. Oliver (Cornell University) is the discovery that the Blue Ridge and Piedmont provinces of the Appalachians are allochthonous and have been thrust at least 275 km westwards.

Appropriately enough, considerable attention was paid to the Wilson Cycle of ocean opening and closure, the principal preoccupation being the character of subduction zones. S. Uyeda (Earthquake Research Institute, Tokyo) presented a model of two contrasted types of subduction zone exemplified by the eastern and western sides of the Pacific, which was developed in more detail by J. F. Dewey (State University of New York, Albany). Flat subduction trajectories produced by flat mantle flow and/or the subduction of young lithosphere produces pervasive compressional strain in the overriding slab at high slip-rates. Steep subduction trajectories, subduction of old lithosphere and low slip-rates induce back-arc spreading. Dewey went on to argue that plate tectonics in its present form has been in operation for about 2×10^9 yr, a view supported by P. Hoffman (Geological Survey of Canada) in his description of an early Proterozoic Wilson cycle in northern Canada.

Similarly, there was accord between Dewey and W. R. Dickinson (Stanford University) that a tectonic mosaic involving horizontal motions was already in existence in Archaean times, and the model of early continental evolution put forward by S. Moorbath (Uni-

versity of Oxford) was consistent with this interpretation. Moorbath argued strongly that isotopic data pointed to continental growth by irreversible differentiation of the upper mantle throughout geological time, rather than by reworking and regeneration of much older crust. Intriguingly enough, Tuzo Wilson was once most widely known for his notion of continental accretion, albeit at a time when he was a staunch opponent of continental drift.

Other aspects of subduction were dealt with by W. S. Fyfe (University of Western Ontario), who argued for substantial subduction of sediments, a view contested by Moorbath, and A. Miyashiro (State University of New York, Albany). Miyashiro concerned himself with metamorphism and estimated that W. G. Ernst's successive models of the high pressure—low temperature zone had greatly improved our detailed understanding, whereas serious problems remained with the low pressure—high temperature zone, notably the origin of the magmas generated there. It seems that the old, widely accepted model assuming magma generation by simple partial melting of the oceanic crust in a descending slab is no longer tenable.

A reassessment of Panagaea reconstructions and time of Mesozoic breakup was undertaken by A. Hallam (University of Birmingham). The familiar 1-km isobath computer reconstructions were inaccurate in detail because it is now known for many regions that the boundary of continental and oceanic crust must occur beneath 2–4 km or even greater depths of ocean, breakup having been preceded by extensive stretching and subsidence. Furthermore, there may be a long time interval between initial taphrogenic activity ('rifting') and creation of ocean floor by spreading ('drifting'). A. J. Boucot (Oregon State University) expressed scepticism about reconstructions of changing continental positions in the Palaeozoic and attempted to show by plotting fossil and climatically significant sediment distributions that an unchanging Panagaea was as satisfactory a model as any. On the other hand, H. Williams (Memorial University, Newfoundland) argued that geological evidence in the Appalachians supported the view that the Iapetus was a major ocean. Problems indeed remain in reconstructing continental positions in the Mesozoic and Cainozoic, but they seem to be child's play in comparison to earlier periods of time. Our best hope would seem to rest with the palaeomagnetists. □

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* The symposium was held at the University of Toronto on 14–16 May 1979 and the proceedings will be published as a special paper of the Geological Association of Canada.

review article

Quantum chromodynamics

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There has been a growing conviction during the past few years within the high-energy physics community that a fundamental theory of the strong interaction has been found. This theory is called quantum chromodynamics (QCD) and if correct it will be a major triumph for theoretical physics. In this review we trace the experimental and theoretical developments which gave rise to QCD, outline its basic properties and discuss its successes and remaining problems.

THE fundamental forces in nature are conventionally divided into four categories. They are, in order of their apparent strengths, strong, electromagnetic, weak and gravitational interactions. Two of these, electromagnetic and gravitational interactions, are long range and hence dominate our physical surroundings; so they are the most familiar and best understood. On the other hand, both strong and weak interactions are extremely short range (they only occur over distances $\leq 10^{-13}$ cm), so their relevance and study are primarily limited to the disciplines of nuclear and elementary particle physics. The weak interaction is responsible for phenomena such as β decay of neutrons into protons and neutrino interactions with matter; in addition it violates some otherwise exact symmetries of nature such as parity and time reversal invariance. The weak interaction gained notoriety recently because of the success of the Weinberg-Salam model^{1,2} in combining weak and electromagnetic interactions into one theory. Finally, there are the strong interactions, which had until recently been poorly understood; but some very significant advances have been made and these are reviewed here.

The strong interaction is primarily known for its role as the powerful short-range force that binds protons and neutrons together inside atomic nuclei. In that capacity, the nuclear binding force is usually described as being due to the exchange of mesons (strongly interacting integer spin particles) such as pions between nucleons. High-energy accelerator experiments during the past several decades have shown that nuclear binding is merely one of many manifestations of the strong interaction. We now know that, in addition to the proton, neutron and light mesons, many other hadrons exist which are produced and interact with one another via the strong force. (The term hadron refers to any strongly interacting particle.) There are evidently an infinite number of hadrons which vary from the familiar stable proton to highly unstable resonances which live for much less than a billionth of a second in the laboratory. The task of a fundamental theory of the strong interaction is to account for all these diverse hadrons and to provide a dynamical description of their properties.

The current view is that the observed hadrons are not elementary particles but are bound states of truly elementary constituents called quarks, and that strong interactions between hadrons are merely epiphenomena of the more fundamental forces between quarks. Of course, the forces that bind quarks together inside hadrons must be extraordinarily strong, as experiments have not revealed an isolated free quark. Furthermore, we now believe that the strong interactions between quarks may be fully described by a quantum field theory called quantum chromodynamics (QCD). We describe here some of the major experimental and theoretical developments which gave rise to the emergence of QCD as a viable theory of the strong interaction.

The quark model

THERE are hundreds of known hadrons (an infinite number if excitation levels are included) which exhibit widely diverse properties. This observed spectrum of hadrons is divided into two classes, mesons which have integer spin (0, 1, 2...) such as the pion (π), kaon (K), and rho (ρ) and baryons which have half-integer spin (1/2, 3/2...) such as the proton (p), neutron (n), lambda (Λ), and omega (Ω). Examination of their specific properties indicates that mesons and baryons can be further subdivided or grouped into multiplets in which the members are all quite similar. This classification scheme is called the eightfold way³; it provides the same kind of organisation for hadrons as the Periodic Table does for chemical elements. It was the regularity of these multiplet groupings that led Gell-Mann and Zweig to conjecture that all hadrons could be built up as bound states of a few fundamental spin 1/2 constituents called quarks. Their conjecture is the basis of the quark model⁴⁻⁷. In this scheme, mesons are bound states of a quark (q) and antiquark ($\bar{q}$), while baryons are constructed from three quarks qqq (antibaryons are made out of three antiquarks $\bar{q}\bar{q}\bar{q}$). (Note that an antiparticle has the same mass as a particle but exactly opposite quantum numbers.) The mesons and baryons so constructed should have integer electric charge, even though their

constituent quarks carry fractional charge. Using these simple rules one can build all the observed hadrons out of quarks. The quark model has been very successful in predicting new hadronic states and correctly describing their properties. It is one of the cornerstones of elementary particle physics.

Five distinct species of quarks, or flavours, are known. In order of increasing mass, the quark flavours are up (u), down (d), strange (s), charm (c) and bottom (b). It is also generally believed that a sixth flavour, the top quark (t), exists at an energy somewhat higher than that now probed by accelerators. The up and down quarks are the lightest; next comes the strange quark which is about 50 times heavier than the up quark. All hadrons discovered before 1974 (the year of the J/ψ particle's discovery^{8,9}) are built up out of these three flavours (u, d, and s); that is why they formed the basis for the eightfold-way classification scheme. Charm and bottom flavours are more recent additions to the quark model. They are respectively about 375 and 1,125 times heavier than the up quark, so their existence could only be uncovered by modern day high-energy accelerators. The top quark's mass is thought to be larger still; there may also be other even heavier flavours waiting to be discovered. All quarks carry fractional electric charge; the d, s and b have charge $-1/3$ (in units of the proton's charge) while the u, c and t (?) have charge $+2/3$. These, with other quark properties, are shown in Table 1.

Table 1 Quark flavours and their properties

Quark flavour	Symbol	Mass ratios*	Charge	Baryon no.	Spin
Up	u	1	$+2/3$	$1/3$	$1/2$
Down	d	2.5	$-1/3$	$1/3$	$1/2$
Strange	s	50	$-1/3$	$1/3$	$1/2$
Charm	c	375	$+2/3$	$1/3$	$1/2$
Bottom	b	1,125	$-1/3$	$1/3$	$1/2$
Top (?)	t	3,375 (?)	$+2/3$	$1/3$	$1/2$

* With respect to m_u ; it is usually estimated that $m_u \sim 4$ MeV.

Within the quark model framework, all known hadrons can be built up out of the five quark flavours u, d, s, c and b (see Fig. 1). For example, the proton is a bound state of one down and two up quarks while the neutron is made out of one up and two down quarks. Similarly, 'strange' hadrons can be made using the s quark. An example of a meson is the recently discovered spin 1 J/ψ particle^{8,9} which is now recognised as being merely one energy level of charmonium, that is a bound state of charm and anticharm ($c + \bar{c}$). Similarly, an even more recent meson¹⁰, the upsilon T , is thought to be an energy level of bottomonium ($b + \bar{b}$).

An assumed property of the strong interactions between quarks is that they are flavour independent and flavour conserving. This accounts then for the strong interaction conservation laws observed at the hadronic level such as isospin, strangeness, and charm conservation. Only weak interactions change the flavours of quarks. They are responsible for flavour changing decays of hadrons such as the beta decays $n \rightarrow p^+ + e^- + \bar{\nu}_e$, and $\Lambda^0 \rightarrow p^+ + e^- + \bar{\nu}_e$.

All our knowledge regarding quarks has been obtained indirectly from the properties of hadrons, as an isolated quark has never been observed. Given the outstanding success of the quark model, why have free quarks never been observed? Are quarks so tightly bound together that they cannot be dislodged from their hadronic domains? The apparent impossibility of liberating quarks has been one of the great puzzles of the quark model.

Deep-inelastic scattering experiments

Although the quark model was very successful in classifying and accounting for the observed hadrons, there was no compelling

evidence that hadrons were actually built up out of quarks until the electroproduction experiments¹¹ were carried out at the Stanford Linear Accelerator Center (SLAC) in 1968. These experiments provided direct evidence that hadrons are really made up out of point-like elementary constituents.

The prototype SLAC experiment consisted of scattering a very high-energy (≈ 20 GeV) beam of electrons on a proton target (see Fig. 2). (The electron (e) belongs to a class of elementary particles called leptons, which can interact electromagnetically and weakly but not strongly. Other known leptons are the muon (μ), tau (τ) and the associated neutrinos ν_e , ν_μ and ν_τ .) As electron-proton scattering proceeds via the electromagnetic interaction as shown by the virtual photon exchange in Fig. 2, it provides a good way of probing the proton's internal structure. If the proton's electric charge were diffused throughout the internal structure, then at very high energies the inelastic scattering cross-section would be expected to decrease very rapidly. That was not observed. Instead, the cross-section decreased slowly in a manner that suggested the proton's electric charge was concentrated in point-like constituents; these were the quarks inside the proton. When high-energy neutrino beams became available, they were also used as hadronic probes and predictions based on the quark model were again confirmed.

A surprising picture emerged from these deep-inelastic scattering experiments. Although the strong interactions of quarks seem to be very complicated at low energies, they become much simpler at high energies. Indeed, at very high energies (or equivalently at very short distances) it was found that quarks behave as though they were essentially free particles inside the hadron. This is called scaling behaviour¹². Contrary to the behaviour of all other known fundamental forces, the strong interaction actually diminishes as one probes nearer the quark. An explanation of this remarkable dynamical property had to await the advent of QCD.

Colour

In the late 1960s it became clear that the simple quark model was inadequate. In addition to flavour, quarks must carry an additional quantum number which has come to be known as colour¹³. Each quark flavour comes in three distinct colours; that is, there are really three times as many quarks as previously

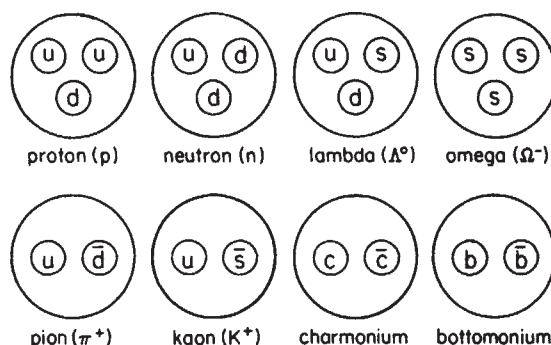


Fig. 1 Pictorial description of the quark content of a few well known hadrons. Inside hadrons, quarks act like free point-like particles.

described. For example, there are red, green and blue up quarks denoted by u_r , u_g , u_b ; they are identical in all respects (mass, charge and so on) except colour. Similarly, the other flavours also come in these same three colours (d_r , d_g , d_b , s_r , s_g , s_b , and so on). Why was this colour degree of freedom necessary?

Colour was originally introduced as a way of reconciling quark statistics with the observed low-lying baryon spectrum. As quarks have spin $1/2$, they should obey Fermi statistics, that is all quantum states should be anti-symmetric (change sign) under

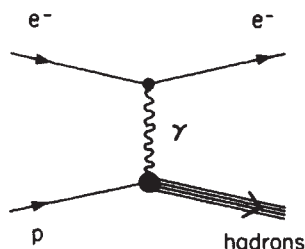


Fig. 2 Deep-inelastic electron-proton scattering proceeding through the electromagnetic interaction. The final state contains many hadrons.

the interchange of two identical quarks. However, this expectation seemed to be violated by the lowest energy baryons. For example, the Ω^- (see Fig. 2) is made up of three spin 1/2 strange quarks; so according to Fermi statistics its wave function should be antisymmetric under the interchange of any two of these quarks. But the Ω^- has total spin 3/2 so the spin part is symmetric; and as it is the lowest energy state of three s quarks, they ought to have zero orbital angular momentum relative to one another, thereby implying that the spatial part of the wave function is also symmetric. However, the total wave function is then symmetric under quark interchange in violation of Fermi statistics. This apparent inconsistency is overcome by giving each of the quarks an additional degree of freedom, colour. Then antisymmetrising the state in the quarks' colour variables renders the Ω^- overall antisymmetric and resolves the problem. The antisymmetrised Ω^- state is denoted by

$$(s_r^1 s_g^2 s_b^3 - s_r^1 s_b^2 s_g^3 + s_b^1 s_r^2 s_g^3 - s_b^1 s_g^2 s_r^3 + s_g^1 s_b^2 s_r^3 - s_g^1 s_r^2 s_b^3) 6^{-1/2}$$

where the superscript labels the three distinct quark wave functions; note that it changes sign if any two quarks are interchanged (for example, $1 \leftrightarrow 2$), as required. In the same way all baryons are made antisymmetric in colour; they have zero net colour. (Our choice of the standard primary colours for the quarks was arbitrary; however, it helps convey the physics, as equal admixtures of these colours yield a colourless state.) Mesons are colourless because they are made from a quark + antiquark which must have opposite colour quantum numbers.

The introduction of colour also resolved other problems, one of which was the decay rate of the neutral pion into two photons, $\pi^0 \rightarrow 2\gamma$. The experimentally observed rate was nine times larger than the quark model's prediction. Introducing colour increased the number of intermediate quark states and the corresponding pion decay amplitude by a factor of 3. In this way the predicted rate was increased by the necessary factor of $9 = 3^2$, which is further convincing evidence for three colours.

Another indication of colour's requirement came from the quantity R which is the ratio of the cross-sections for electron-positron annihilation into hadrons and muons, $R \equiv \sigma(e^+e^- \rightarrow \text{hadrons})/\sigma(e^+e^- \rightarrow \mu^+\mu^-)$. Without colour, the experimental value of R was three times larger than the quark model's prediction. Introducing a factor of 3 for colour brings them into agreement. The three pieces of input, baryon statistics, $\pi^0 \rightarrow 2\gamma$ decay rate and the value of R are the best available evidence for colour.

Why is this additional quantum number called colour? The nomenclature colour provides a convenient way of describing the fact that the observed hadrons do not carry this new quantum number, they must be colourless. It is equivalent to the mathematical statement that each quark flavour transforms as a triplet representation under an internal $SU(3)_c$ colour symmetry; but physical hadron states are all singlets.

The quark model when appended with the $SU(3)_c$ colour symmetry provides a good description of the observed hadronic spectrum. However, it fails to account for the dynamics of the strong interaction and leaves unanswered some obvious questions. Why do quarks inside hadrons behave as though they were free when we probe them in very high-energy experiments or

equivalently, why does the strong interaction's strength decrease in the vicinity of quarks? Why are free quarks or any other coloured hadronic states not observed in nature? QCD answers the first question and, we believe, may also answer the second one.

Gauge theories

Quantum chromodynamics is a particular example of a general class of theories called non-abelian gauge theories. Significant developments in our understanding of these theories have occurred during the past decade which set the stage for QCD's emergence. Some of these advances will be outlined after we have briefly explained the fundamentals of gauge field theories.

The basic idea of a gauge theory is that a continuous symmetry or global invariance property of a lagrangian field theory can be made into a local invariance by introducing compensating gauge fields into the theory. This means that given a field theory which possesses a symmetry such as $U(1)$, $SU(2)$, $SU(3)$ or any other Lie group, the theory can be extended to a gauge theory which has that symmetry at each point in space-time individually. The new symmetry is called a gauge symmetry because it implies that we can choose our measuring standard (gauge) (such as, $SU(2)$ isospin basis) differently throughout space-time without changing the physics of the theory.

The most familiar example of a gauge theory is electromagnetism. In that case the symmetry group is $U(1)$ corresponding to the freedom of changing the phase of any electrically charged field ($\psi(x) \rightarrow e^{i\theta}\psi(x)$); a symmetry that follows from charge conservation. Extending this to a local $U(1)$ gauge invariance ($\psi(x) \rightarrow e^{i\theta(x)}\psi(x)$) requires the introduction of a single gauge field, the electromagnetic vector potential $A_\mu(x)$, which becomes the photon field in the quantum theory.

Quantum electrodynamics (QED), the quantum field theory of the electromagnetic interactions of charged particles and photons, is our most successful gauge theory. It is renormalisable, which means that all short distance (ultraviolet) divergences encountered in perturbation expansions can be absorbed into the definitions of the physical parameters (electric charge, masses, and so on); in terms of these quantities all predictions of the theory are finite. QED's predictions for precisely measured quantities such as the anomalous magnetic moments of the electron and muon and the Lamb shift in hydrogen are in perfect agreement with experiment. The incredible accuracy of QED strengthens our belief that renormalisable theories and gauge theories in particular, may also provide good descriptions of the other fundamental interactions.

The generalisation of the principle of local gauge invariance from the electromagnetic $U(1)$ group to an arbitrary Lie group was accomplished by Yang and Mills¹⁴ in 1954. Their work showed that the number of required compensating gauge fields equals the number of group generators and that in the case of

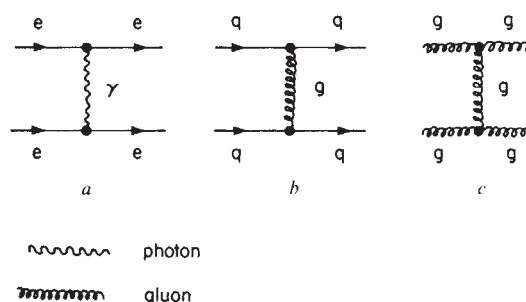


Fig. 3 Diagrams depicting: a, electron-electron electromagnetic interaction via the exchange of a virtual photon; b, quark-quark strong interaction via the exchange of a virtual gluon; c, gluon-gluon strong interaction via the exchange of a virtual gluon. c illustrates the essential difference between QED and QCD (photons do not self-interact); this novel feature gives rise to asymptotic freedom and perhaps colour confinement as well.

non-abelian groups (SU(2), SU(3) and so on) the gauge fields interact among themselves; a novel feature with extraordinary consequences.

Non-abelian gauge theories lay dormant until two theoretical advances were made. The first was the development of a procedure for quantising non-abelian gauge theories by Faddeev and Popov¹⁵. It elevated them from classical field theories to quantum field theories in which the interacting fields could be identified with particles (quanta). The second was a formal proof of the renormalisability of non-abelian gauge theories which came from the work of 't Hooft and Lee and Zinn-Justin^{16,17} in 1971–73. This put non-abelian gauge theories on as firm a theoretical footing as the successful QED, and made them potential candidates for describing the other fundamental interactions.

An immediate outgrowth of the work just described was renewed interest in the Weinberg–Salam model^{1,2} of weak and electromagnetic interactions which is based on the gauge group SU(2) × U(1) (often called quantum flavourdynamics (QFD)). This theory describes and unifies all known weak and electromagnetic phenomena; it has been very successful in predicting now observed neutrino neutral current scattering cross-sections¹⁸ and parity violating asymmetries in deep-inelastic polarised electron scattering¹⁹. These successes provide objective evidence that non-abelian gauge theories do describe physical phenomena.

Asymptotic freedom

In 1973 a remarkable property of non-abelian gauge field theories was uncovered by Politzer and Gross and Wilczek^{20,21}. They found that because of the self interactions among gauge fields, these theories exhibit the property of asymptotic freedom. That is, the strength of the interactions mediated by non-abelian gauge fields becomes vanishingly small at asymptotically high energies (or equivalently at very small distances). These theories behave almost like free field (non-interacting) theories in the asymptotic energy regime. This was precisely the property observed in the deep-inelastic scattering experiments: at high energies quarks inside hadrons behave as though they were free. Furthermore, it was shown that non-abelian gauge field theories are the only field theories which exhibit asymptotic freedom²². An obvious conclusion was that the strong interactions between quarks must be mediated by non-abelian gauge fields.

Quantum chromodynamics emerges

The asymptotic freedom of non-abelian gauge theories fits beautifully with the quark model of hadrons and the requirement for three hidden quantum numbers called colour; from these distinct ideas and discoveries quantum chromodynamics (QCD) emerged (QCD is reviewed in ref. 23). The basic ingredients of QCD are the following: each quark flavour (u, d, s, c, b, and so on) comes in three colours (for example, red, green, and blue) which differentiate what would otherwise be three identical quarks. The three colours of quarks (u_r, u_g, u_b) transform as a fundamental triplet of the group SU(3)_c where the subscript c stands for colour. This SU(3)_c symmetry is an exact symmetry of nature; it is never violated. The key new idea of QCD is that the SU(3)_c symmetry must be a local gauge symmetry. This extension to a local SU(3)_c gauge symmetry requires the introduction of eight vector gauge fields called coloured gluons (corresponding to the eight generators of the SU(3)_c group) which transform as an SU(3)_c octet. The gluons are massless spin 1 quanta which mediate the strong interaction between coloured quarks (see Fig. 3). In that way, gluons are to QCD what photons are to QED; however, an extremely important difference is that gluons carry colour and therefore interact strongly among themselves, while photons are electrically neutral and therefore cannot directly interact with one another. This distinction gives rise to asymptotic freedom in QCD (the effective coupling between

fields decreases at short distances) and perhaps other dramatic consequences as well.

The flavour quantum number of the quarks plays no role in the QCD strong interaction; all flavours of quarks interact strongly with one another in the same manner. Colour interactions are flavour blind while weak and electromagnetic interactions are colour blind.

The immediate success of QCD was that it incorporates all the earlier successes of the quark model with colour. Furthermore, this quantum field theory has all the observed strong interactions symmetries, flavour conservation, parity and time reversal invariance, and no additional unobserved symmetries. Also, if a theory of the weak and electromagnetic interactions such as the Weinberg–Salam model is appended to QCD it does not disturb these strong interactions selection rules except by small, calculable amounts, nor do the strong interactions disturb weak and electromagnetic phenomenology.

One can go beyond symmetries to real dynamics. The property of asymptotic freedom inherent to this exact non-abelian gauge theory accounts for the scaling phenomena observed in deep-inelastic electron and neutrino scattering experiments. It explains why at very high energies quarks act as though they were free particles inside hadrons. Furthermore, we now know that the scaling behaviour is not exact. There are small logarithmic deviations from exact scaling predicted by QCD and they are experimentally observed, a beautiful confirmation of the theory²⁴.

The fact that QCD's strong interaction gauge coupling constant decreases as the energy domain probed increases (which follows from asymptotic freedom) allows one to calculate high-energy QCD effects perturbatively in this small coupling. Today an enormous effort is underway to compute QCD predictions for lepton–hadron and hadron–hadron scattering processes using perturbation theory. One observed effect that perturbative QCD accounts for nicely is the jet structure of final state hadrons produced in electron–positron annihilation ($e^+e^- \rightarrow \text{hadrons}$) at high energies—the tendency of hadrons to be produced with very little transverse momentum in streams of

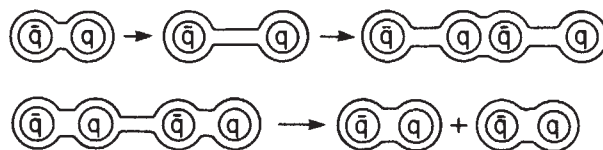


Fig. 4 An unsuccessful attempt to free a quark from a bound state meson. The energy expended in pulling the quarks apart goes into creating a quark–antiquark pair out of the vacuum and thereby leads to two colourless mesons. Quarks and their colour remain confined.

narrow jet-like cones²⁵. In principle it is possible to calculate QCD predictions perturbatively to arbitrary precision in such high energy kinematic regimes and compare with experiment; thereby rigorously testing the viability of QCD.

Another experimental-consistency check of QCD involves bound state mesons of heavy quark flavours. Heavy quark systems are a good test for QCD because the binding interaction is at short distances, so the coupling is diminished and perturbation theory becomes applicable. For example, charmonium which is a bound state of c and $\bar{c}$ quarks has been vigorously investigated using QCD (charmonium is reviewed in ref. 26). In this way, the observed energy level splittings and various partial decay rates of charmonium have been reasonably well explained. The more recently discovered upsilon T, which is a bound state of b and $\bar{b}$ quarks, should provide an even better system for investigating QCD effects as the coupling strength for that very massive quark system is even smaller.

Using QCD one should eventually be able to calculate hadron masses, the couplings between mesons and baryons, strong interaction cross-sections, essentially all strong interaction

phenomena. At present, the optimism surrounding QCD results from the fact that it is not contradicted where it can be checked experimentally. We can expect QCD to be subjected to more rigorous tests in the future.

There are still some outstanding problems confronting the QCD field theorist, the foremost being to prove the absolute confinement of colour. Because quarks, gluons and coloured bound states have never been observed, colour must be somehow confined (not physically observable) in QCD. It is believed at present that as one tries to break a colourless hadron apart into its coloured constituents, the potential energy grows linearly until there is enough energy to create new quark-antiquark pairs and gluons out of the vacuum. In this way the hadron can divide into several colourless hadrons, so that quarks, gluons and their colour remain confined (see Fig. 4).

One way that this scenario might be realised in QCD is through 'infrared slavery'—the opposite of asymptotic freedom. Infrared slavery means that the self-interaction of non-abelian gauge fields causes the inter-quark and -gluon couplings to become very large at large distances (perhaps infinite). This accounts for strong quark binding and might, hopefully, imply colour confinement.

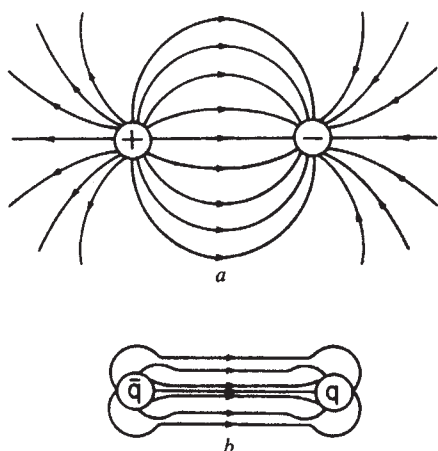


Fig. 5 *a*, Electric field lines between positive and negative electric charges. *b*, Sketch of the anticipated colour field lines between a quark and antiquark. The lines in (*a*) give rise to a Coulombic potential $\sim 1/r$, and those in (*b*) give rise to a linear confining potential $\sim r$.

Another possibility is that the colour gluon fields may form a flux tube or string between colour sources. If the coloured flux in the tube is conserved it is easy to show that the potential energy between sources rises linearly with the separation, implying confinement of colour (see Fig. 5).

Another idea is that the vacuum state surrounding a hadron is a very complicated strong coupling regime. The hadron is a little bag or bubble in this vacuum describing a two-phase system. The quarks and gluons inside are in relatively free motion. Only if the quarks or gluons try to escape from the bag do they experience strong confining forces. This is essentially the phenomenologic-

ally successful MIT bag model (see the review in ref. 27). The problem is to show that such a bag picture of hadrons actually follows from QCD.

There are other problems besides that of the colour confinement. The pion (π) seems experimentally to be a collective excitation of quarks rather than an atomic bound state. This property of pions can be understood as the spontaneous breaking of the right-left symmetry of the quarks (chiral symmetry). The problem is to show that in QCD chiral symmetry is actually spontaneously broken.

A new class of gauge field configurations, instantons²⁸, have recently been discovered in the classical non-abelian gauge theories and they may provide a better understanding of the quantum theory (for example, QCD). In the quantum theory, instantons imply that tunnelling takes place between topologically distinct vacuum states. There are already suggestions that instantons may be responsible for breaking chiral symmetry in QCD thus elucidating the pion's properties. Instantons may also lead to a two-phase structure in QCD—strong and weak coupling—that could give rise to a bag picture of confinement²⁹. Many theorists are trying to solve these problems and elucidate QCD's properties.

Outlook

QCD and QED (Weinberg-Salam model) are both non-abelian gauge theories. Together they describe strong, weak and electromagnetic interactions. The next natural step is to embed these two distinct theories in a larger compact covering Lie group, for example, SU(5) (ref. 30), and thereby construct a grand unified theory of strong, weak and electromagnetic interactions. Such a theory has only one gauge coupling constant, so all three interaction strengths are equal at very short distances (true unification); however, this unification only becomes apparent at ultra-high energies $\sim 10^{16}$ GeV. In these types of models leptons and quarks belong to the same group representations. So leptons, such as the electron, are very much like quarks; but they are not strongly interacting and are liberated because they do not carry colour. A novel prediction of many such grand unified models is that baryon number is not absolutely conserved; so the proton may actually decay! (Its half life is $\tau_p \geq 10^{30}$ yr.) This predicted instability is being looked for experimentally. Ultimately, one wants to bring gravity into this unification scheme; however, in spite of much effort a renormalisable quantum theory of gravity is not known to exist.

For decades high energy theorists have sought a fundamental theory of the strong interaction: many are now convinced that QCD is the answer. It can be explicitly written down as a renormalisable quantum field theory; the problem has become to solve QCD and make further experimental contact. The endurance of the concept of a continuum quantum field theory as a way of understanding physics at 10^{-13} cm and smaller is especially remarkable.

The shift in emphasis from searching for the theory of the strong force to solving it may revitalise the symbiosis of mathematics and physics in the coming decades. Strong interaction physics could become like atomic physics after the Schrödinger equation.

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articles

Re-appraisal of lithostratigraphy of Makapansgat Limeworks hominid site

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A new lithostratigraphic classification is proposed for the Makapansgat Limeworks hominid site. The Makapansgat Formation is defined in terms of constituent members and beds. The new terminology is correlated with that used previously and hominid finds are linked to the newly defined lithostratigraphic units.

THE stratigraphy of the hominid site at Makapansgat Limeworks, where *Australopithecus africanus* was discovered in association with a rich fauna in 1947, has been described in several publications. Between 1947 and 1957 several broad descriptive classifications were proposed¹⁻⁵. The first detailed stratigraphic and sedimentological analysis appeared in 1958 (ref. 6) and has provided the standard lithostratigraphic subdivision of the deposit until recently. The re-appraisal of the hominid deposit reported here was necessary because earlier studies omitted several important subdivisions within major stratigraphic units. In this re-appraisal I call the hominid-bearing deposit the Makapansgat Formation.

Location and origin

The Makapansgat Formation comprises a large cave filling at the Makapansgat Limeworks (24°08' S, 29°11' E). It is exposed both at surface and abandoned lime quarries at elevations of 1,440–1,475 m. At the surface the maximum dimensions of the deposit are approximately 200 × 115 m, increasing slightly with depth. The outcrop area is associated with a pronounced bench on the steep southeastern slopes of the Dorps river valley.

The deposition and subsequent modifications of the sedimentary sequence were very similar to those at the Sterkfontein hominid site, described previously⁷. A similar origin, conforming to the model of karst development proposed for the Transvaal⁸, is postulated for the original solutional receptacle at Makapansgat. The Malmani dolomite here dips south at a low inclination; together with joints and small faults, this small dip caused preferential solution and influenced the form of the original cavern⁸. Considerable modifications resulted from later roof collapses. The present water table is controlled by the episodic Dorps river, and is usually below the level of the valley floor, ~15 m below the base of the formation.

Small remnants of the original dolomite roof are preserved over the deposit, but much of its surface has been bevelled by erosion of the hillside. Reconstructions based on the positions and inclinations of the roof segments suggest that the maximum elevation of the original roof reached ~20 m above the present surface. Hence the absolute thickness of the deposit may have approached 50 m in places; less than 60% of this is preserved.

As with the Sterkfontein Formation⁷, the stratigraphic relationships are complex, and the schematic column in Fig. 1 merely reflects the relative average thicknesses of the various units in accessible areas of the deposit.

Lithostratigraphy

Samples were prepared in the same way as those from Sterkfontein⁷. They were recovered in vertical succession from four areas at an average vertical separation of <2 m; supplementary samples were recovered from other localities to monitor lateral variations in sedimentary facies.

The very coarse (>2 mm) fraction of all units was examined visually. In the lowest members (1–3) it is chiefly bone and broken speleothems, although occasional pebbles and cobbles of dolomite, chert, quartzite and shale occur in Member 3. In Members 2 and 3 much of the bone is stained with pyrolusite, and many of the broken speleothems show post-depositional weathering. In Member 4 the very coarse fraction is predominantly angular cobbles and boulders of chert and dolomite; many individual fragments are fresh on one or two faces with thin weathering skins on others, indicating that they accumulated through intracavernous collapses of the roof and projecting ledges. The finer component includes subrounded chert pebbles and cobbles whose deeper and more uniform weathering is compatible with an extracavernous colluvial source. Occasional weathered quartzite pebbles and cobbles are present. Quartzite and shale horizons are interbedded in the Malmani dolomite on the upper valley slopes to the south-east of the site.

The very coarse fraction of Member 5 consists overwhelmingly of deeply weathered subangular to subrounded dolomite pebbles and cobbles with few chert and quartzite pieces.

Member 1 is exposed over most of the cave above the irregular dolomite floor, or overlying large collapsed roof-blocks. It consists of 0.5–15.0 m of white banded reddish-brown (5 YR 5/4), recrystallised meso- and macro-crystalline calcite, locally contaminated with silty loam (clayey, sand-silt). It is thickest in two major stalagmitic bosses, one aligned north-south through the Entrance Quarry (see Fig. 2), the other along the south-east wall of the cave. Concentrations of bone, sometimes as articulated partial skeletons, are present in the distal areas; occasional moderately inclined centimetre beds and cross-beds are visible. The evidence suggests that extracavernous components entered through a relatively small opening near the northern end of the cave. CaCO₃ generally exceeds 80% and average ϕ mean is 6.0 (ϕ is the negative logarithm to the base 2 of the particle diameter (mm)). Sediments are poorly sorted⁹ and leptokurtic or highly leptokurtic with pronounced positive skewness. Crystalline minerals (in decreasing order of abundance) are quartz, kaolinite and sericite. Typical particulate roundness and sphericity are 0.3 and 0.5 respectively, and conform with those of present external colluvial soils. As in most succeeding units, quartz particles predominate; chert and dolomite grains are infrequent. Pyrolusite and ferric skins are present on a few particles. Contact is abrupt and wavy.

These features suggest episodic subaqueous deposition of the clastic material with illuvial enrichment in fines. Cross-stratification indicates locally channelled flow during episodic

storms, with relatively high water velocities occurring away from high points in the floor near the centre and entrance of the cave. These could have swept carcasses from the entrance into distal recesses.

Member 2 is exposed to the west and south-west of both the Entrance and the Exit Quarries, where sedimentation occurred penecontemporaneously in two separate depositories separated by a high area in the surface of Member 1. It consists of 2.0–10.0 m of yellowish-red (5 YR 5/8), and, less frequently light to medium red (2.5 YR 6/6 and 4/8) or light reddish-brown (5 YR 6/4), mostly centimetre-bedded and cross-bedded, silty loam (clayey sand-silt), containing frequent broken speleothems, recrystallised calcite lenticles, and with local concentrations of bone, including some articulated partial skeletons in distal areas. Pebble-sized rolled calcite and bone fragments have sometimes been retained in irregularities in the inclined surface of Member 1 near the position of the original cave opening, and marked lateral changes of facies are evident. Occasional lenses of spongy collophanite and oolitic concretions occur near the top of the member, which has a very uniform upper surface at approximately 1,458 m. CaCO_3 averages 56% and average ϕ mean is 5.3. Sediments are poorly to very poorly sorted and mesokurtic to leptokurtic and samples have a wide range of skewness from negative to positive. Crystalline minerals are quartz, sericite and haematite. Typical roundness and sphericity are 0.1 and 0.5, indicating higher angularity than is characteristic of the present external colluvial soils. A few particles have pyrolusite and ferric skins. Contact is eroded, slightly wavy and abrupt, and truncates the uppermost cross-beds. The sedimentology indicates deposition of this member beneath the fluctuating surface of a water table which reached about 1,458 m, with local flushing of some fines into more distant depositories. Fairly widespread cross-beds with rolled calcite pebbles and bones in the proximal facies indicate channelled flow with relatively high water velocities down the irregular, sloping surface of Member 1. As in Member 1, these flows could have carried carcasses into recesses. The collophanite lenses indicate significant bat populations towards the end of this deposition.

Member 3 is exposed at the southern end of the cave to the east and west of the Cone area, where it consists of 0.5–2.0 m of abundant bone fragments (some semi-articulated) in a matrix of very pale brown (10 YR 8/3 and 8/4) and brownish-yellow (10 YR 6/6), sometimes blotched reddish-brown (2.5 YR 5/4) and black, sandy to silty loam (slightly clayey silty sand), containing extensive calcite intergrowths, numerous broken speleothems, occasional pebbles of dolomite, chert, quartzite and shale, carrion fly pupae, a few coprolites and frequent pockets of collophanite. Most of the deposition occurred in the stalactite fringe immediately below the dolomite cave roof, and crude stratification is present within the bone fragments between stalactite clusters. Flow diversion around the latter has increased bed-shear and created extensive bone lags showing variable particle orientations. CaCO_3 averages 67% and average ϕ mean is 4.6. Sediment samples show much variation, but are generally very poorly sorted and platykurtic to leptokurtic with a wide range of skewness from negative to positive. Crystalline minerals are quartz, fluorapatite, sericite and chlorite. Typical roundness and sphericity are 0.3 and 0.5, and a very few particles have pyrolusite skins. Comminuted bone and chert particles are relatively abundant. Where Member 3 is present vertically below Member 4, for example east of the Cone, it is overlain by some 0.5 m of laminated, recrystallised meso- and macro-crystalline calcite. Contact between Member 3 and calcite is slightly wavy and abrupt.

The characteristics of Member 3, which has produced the majority of the fauna and hominid remains, are consistent with rapid alternations in depositional conditions with the occurrence of several short, high-energy cycles, which coincide with large fluctuations in concentration of the isotopes ^{13}C and ^{18}O in the calcite fraction of the matrix¹⁰. Quieter episodes apparently alternated with vigorous flows which carried bone fragments and

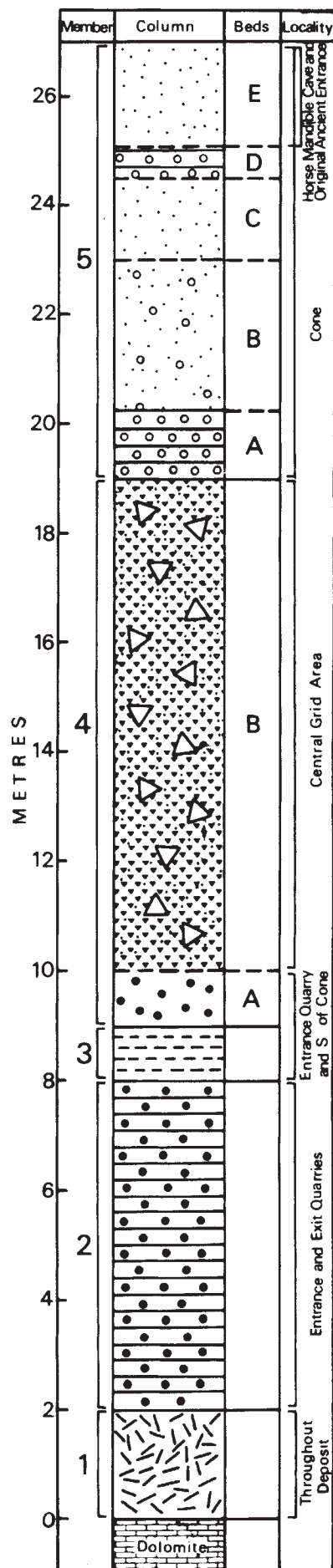


Fig. 1 Stratigraphic column of the Makapansgat Formation.

dismembered carcasses towards the back of the cave and winnowed some finer particles. Between sheetfloods decomposing carcasses attracted carrion flies. The restricted roof height and distance from the cave opening argue against any lengthy occupation of this area by predators or scavengers; such creatures probably concentrated their activities near the cave mouth. Their coprolites could have been incorporated in the deposit during episodes of quieter flow.

Member 4 consists of beds A and B. Bed A is exposed chiefly in the Entrance Quarry and is composed of 0.5–2.0 m of pink (5 YR 7/4) or light red (2.5 YR 6/6), locally decimetre-bedded, silty loam (clayey silty sand), containing numerous large oolitic concretions, occasional recrystallised calcite lenticles and infrequent angular chert and dolomite pebbles and cobbles; some bones and carrion fly pupae are present. Towards the northern end of the Exit Quarry it takes the form of a dense accumulation of rodent bones in a similar fine matrix, which indicates the presence of local owl roost. CaCO_3 averages 92% and the average ϕ mean is 4.65. Sediments are very poorly sorted and platykurtic to leptokurtic with negative skewness. Crystalline minerals are quartz and sericite. Typical roundness and sphericity are 0.3 and 0.5. Pyrolusite skins are rare. Contact is gradational. These features argue for the deposition and redistribution of sediments in an environment of shallow, localised pools accompanied by winnowing of some fines.

Bed B is present throughout the central part of the cave; it is the most extensive stratigraphic unit, and lime-workings have almost everywhere isolated it from the cave walls. Over substantial areas where the cave floor is high, it overlies either Member 1 or Member 2. It is composed of 2.0–15.0 m of light reddish-brown (5 YR 6/4), less frequently pink (5 YR 7/3), reddish-yellow (5 YR 6/6) or red (2.5 YR 5/8), loam (clayey silty sand), containing abundant broken speleothems, chert and dolomite pebbles, cobbles and boulders, a few quartzite pebbles, occasional bone fragments and recrystallised calcite lenticles and void linings. The frequency of chert and dolomite fragments increases upwards, and these display crude stratification parallel to the flanks of a debris cone with its apex near the northern end of the deposit. Highest bone concentrations occur along the cone periphery near the cave walls, suggesting that bones were redistributed toward its margins. CaCO_3 averages 86% and average ϕ mean is 4.95. Sediments are extremely poorly sorted and samples are mostly mesokurtic, with a wide range from platykurtic to leptokurtic. There is a similar range in skewness from negative to positive, with the latter predominating. Crystalline minerals are quartz, sericite and weakly crystallised montmorillonite. Typical roundness and sphericity are 0.3 and 0.7, indicating greater rounding than is characteristic of present external colluvial soils. Pyrolusite and ferric skins are rare. Contact is eroded and disconformable and is locally complicated by subsidence movements.

The characteristics of this bed are consistent with redistribution of gravelly colluvium and some aeolian material, in admixture with frequent roof collapse debris, down the flanks of a cone of gravitative accretion under the influence of episodic sheetfloods entering through an enlarged cave opening. Higher energy conditions are evident towards the middle of this unit.

Member 5 consists of beds A–E. All are exposed in conformable sequence around the margins of the Cone. The uppermost unit (bed E) also occurs in isolated pockets to the west of the Entrance Quarry, in the eroded surface of Member 4, and in Horse Mandible Cave. In the Cone area deformation and displacement of an underlying calcite horizon indicates that Member 5 accumulated in a large depression formed by subsidence of underlying deposits into lower cavities; it is likely that similar movements preceded sedimentation in Horse Mandible Cave. Beds A, B and D are gravelly and are often lenticular in form, grading laterally into sandy facies. The latter often preserve carrion fly pupae.

Bed A consists of 1.0–2.0 m of yellowish-red (5 YR 5/6), silty loam or loam (clayey silty sand), containing abundant subangular or subrounded pebbles and cobbles of partially weathered

dolomite, occasional chert and quartzite pebbles, and a few badly damaged bone fragments. Good sub-horizontal stratification is present in the very coarse fraction. A single sample had a CaCO_3 content of 69% and a ϕ mean of 5.1. The sediments are extremely poorly sorted and mesokurtic with small positive skewness. Crystalline minerals are quartz, sericite, haematite and kaolinite. Typical roundness and sphericity are 0.3 and 0.7, and pyrolusite skins are uncommon. Contact is sloping and fairly abrupt.

Bed B includes 3.0–6.0 m of pink (5 YR 7/4) becoming red (2.5 YR 5/6) where decalcified, unbedded loamy sand (slightly silty sand), containing abundant subangular to subrounded pebbles, cobbles and boulders of partially weathered dolomite, a few chert and quartzite pebbles, and occasional badly damaged bone fragments. CaCO_3 is about 73% where calcified, but falls to about 50% where calcite cement has been removed by solution; average ϕ mean is 2.1. Sediments are very poorly sorted and mesokurtic with pronounced positive skewness. Crystalline minerals are quartz, feldspar, sericite and kaolinite. Typical roundness and sphericity are 0.3 and 0.5 and pyrolusite and ferric skins are rare. Contact is even and abrupt.

Bed C consists of 2.0–10.0 m of red (2.5 YR 5/8), decimetre-bedded loamy sand (slightly silty sand), with a negligible very coarse fraction. Bones are few and badly damaged. A single sample had a CaCO_3 content of 39% and a ϕ mean of 2.6. Sediments are very poorly sorted and mesokurtic with pronounced positive skewness. Crystalline minerals are quartz, sericite and haematite. Typical roundness and sphericity are 0.3 and 0.5 and pyrolusite skins are rare; ferric skins are slightly more abundant. Contact is even and abrupt.

Bed D comprises a distinct near-horizontal gravel band which lenses out and bifurcates locally. It consists of up to 1.0 m of yellowish-red (5 YR 5/6), silty loam (slightly clayey silty sand), containing abundant subangular to subrounded pebbles and cobbles of partially weathered dolomite, occasional chert and quartzite pebbles, and a few badly damaged bone fragments. The very coarse elements show good sub-horizontal stratification. A single sample had a CaCO_3 content of 56% and a ϕ mean of 4.05. The sediments are very poorly sorted and platykurtic with negative skewness. Crystalline minerals are quartz, sericite and haematite. Roundness and sphericity and the presence of skins are akin to those in Bed C. Contact is even and abrupt.

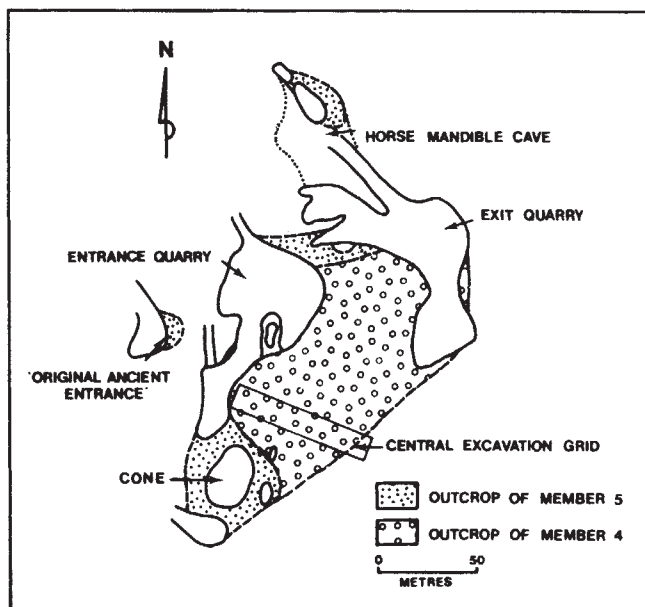


Fig. 2 Locality plan showing the surface extent of the Makapansgat Formation and the positions of localities mentioned in the text.

Table 1 Comparative terminology of Makapansgat Formation

Previous terminology	Present terminology	Hominid remains
(Brain ⁶) Phase II (red sandy and gravelly) breccia	Member 5	—
Upper Phase I (pink stoney) breccia	Bed A and lower bed B of Member 5 and Member 4	MLD 37/38 from bed B of Member 4
Lower Phase I Grey bone-rich breccia	Member 3	Remainder of specimens of <i>A. africanus</i>
Red calcified muds	Member 2	—
Contaminated travertine	Member 1	—

Bed E consists of 1.0–5.0 m of red (2.5 YR 4/6 and 5/6), and less frequently yellowish-red (5 YR 5/8) and reddish-brown (5 YR 5/4), decimetre-bedded sandy loam (slightly clayey silty sand), with a small very coarse fraction of the type present in the remaining beds, and occasional recrystallised calcite lenticles. CaCO₃ averages 65% and average ϕ mean is 3.8. Sediments are very poorly sorted and samples show a wide range of kurtosis from platykurtic to leptokurtic, but are almost all negatively skewed. Crystalline minerals are quartz, feldspar and sericite. Typical roundness and sphericity are 0.3 and 0.7, indicating greater rounding than is characteristic of the present external colluvium. Pyrolusite and ferric skins are somewhat more numerous than in previous beds.

Viewed as a sequence, the five beds of Member 5 suggest deposition by rapid episodic flushing of unconsolidated gravelly soil from the hillside into depressions containing impermanent pools. The presence of a large proportion of subrounded, weathered dolomite fragments suggests that this gravelly material may have been an alluvial terrace or a mudflow deposit, which had undergone a degree of post-depositional weathering before being washed into the cave. However, the fine matrix certainly includes a large colluvial component. The cave openings seem to have been greatly enlarged after deposition of Bed A. Illuviation of fines from upper to lower beds is evident, suggesting relatively slow and inefficient calcification after cessation of sedimentation. There is distinct evidence for an energy increase in Beds B and C, but damage to bone fragments in conjunction with the presence of stratified gravel lenses suggests the existence of high energy levels during deposition of most of the member. The morphology of Bed B, with its conspicuous lack of stratification indicates a rapid mass movement following renewed subsidence during its accumulation.

Extracavernous source materials

Comparative analyses of deposits from the vicinity indicate that the <2 mm fraction of the various units was derived mostly from extracavernous colluvial sources except in Member 5, where a significant alluvial (?) component is evident, especially in the very coarse fraction. The contribution of the insoluble residue produced by intracavernous corrosion is chiefly restricted to a few highly angular chert particles.

The present colluvium has relatively uniform properties; it is a pebbly, skeletal soil containing abundant, partially weathered, chert pebbles and cobbles. The characteristics of the Makapansgat Formation can be explained in terms of the reworking of this material. It is a dark reddish-brown (5 YR 3/3 and 3/4) sandy loam (slightly clayey silty sand) containing abundant weathered chert pebbles and cobbles, and seldom exceeding 50 cm thick. The ϕ mean averages 2.8. The material is very poorly sorted and is mesokurtic and samples show a wide range of skewness, both negative and positive. Crystalline minerals are quartz, sericite and kaolinite. Typical roundness

and sphericity are 0.3 and 0.5 and many particles have pyrolusite skins.

In Member 5, while a large colluvial component seems to be present in the fine matrix, the very coarse fraction has many of the characteristics of an alluvial or mudflow deposit.

Sedimentary facies of the various units of the Makapansgat Formation can be explained through the reworking of extracavernous colluvium by a limited suite of transportational and depositional processes. The colluvium contributing to Member 1 has been enriched in fines, while that of Member 2 shows that, although fines have increased overall, as expected with subaqueous deposition at a distance from the cave entrance, some winnowing has taken place, especially in proximal facies produced by rapid channelled flow. Winnowing of fines is even more evident in Member 3, where it seems to have occurred during several cycles of flushing. The source material for Bed A of Member 4 seems to have been subjected to similar, but probably less intense flushing. In Bed B of Member 4 enrichment in fines is widely evident, possibly due to an aeolian component, although local winnowing can be discerned away from the walls of the depository. Some enrichment in fines, evidenced by pronounced positive skewness, has occurred in beds A–C of Member 5, probably due to post-depositional illuviation from the upper beds before calcification of the deposit.

In general, fine sedimentary fractions are more abundant in lower members, due to a greater degree of illuviation with increasing distance from the cave opening. Removal of fines can be attributed to flushing and eluvial effects as sheetfloods spilled into the cave opening(s) and were channelled downwards into more distant caverns.

Extensive hiatuses in clastic deposition within the cave, sometimes associated with calcite accretion, indicate cycles with reduced influx of extra-cavernous sediments. In some instances such episodes were marked by intra-cavernous scouring and the production of erosional unconformities.

Solution cavities and discrimination between members

Solution cavities, often exceeding 5 m in depth, were formed in the eroded surface of the Makapansgat Formation chiefly in Bed B of Member 4. These contain residual material, produced by decalcification and *in situ* weathering of the surrounding sediments; this is overlain by recent colluvium with a clear transition. The residual material is occasionally bone-bearing, and is similar in its properties to that occurring in solution cavities in the Sterkfontein Formation⁷.

Sedimentological analysis of samples from the various lithostratigraphic units of the Makapansgat Formation reveals that successive members are distinctive in terms of the standard deviation of at least one of the associated quartile and moment measures or of the sedimentological characteristics of their very coarse fractions.

Comparison with Sterkfontein and Swartkrans Formations

The stratigraphies of the Sterkfontein and Swartkrans hominid sites, ~250 km to the south-west, have been re-appraised recently^{7,11,12} and the Sterkfontein and Swartkrans Formations have been defined. Comparison of these formations with the Makapansgat Formation shows that, although similarities exist, each is distinctive in terms of the sedimentology of specific constituent members and its particular sequence of erosional and depositional events. The fauna thus far recovered from Members 1–4 of the Makapansgat Formation has no equivalent at Swartkrans,¹³ but is considered broadly similar to that from the Sterkfontein Type Site (Member 4)^{13–14}, although no results have as yet been published which distinguish comprehensively between the faunas present in the various subdivisions of the Makapansgat Formation; these clearly span a substantial period. However, Wells¹⁶ believes that important differences

between the Sterkfontein and Makapansgat faunas suggest that that from Makapansgat (mostly the lower members) is the older. Palaeomagnetic evidence from the Makapansgat Formation^{17,18} permits the fairly definite conclusion that Member 1 predates 3.32 Myr, while Member 3 predates 2.90 Myr. Faunal age estimates for the Sterkfontein Type Site fall in the range 2 to 3 Myr. More precise correlations between the faunas of the two sites must await further studies of the respective assemblages.

Correlation with previous terminology and hominid finds

In Table 1 the published stratigraphic terminology of previous workers at Makapansgat Limeworks is cross-referenced to the terminology used here. The table also gives an indication of the known or likely provenance of the hominid remains.

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Excavations undertaken so far have sampled very small volumes of the *in situ* deposits, the remaining faunal and hominid material having been extracted from material discarded on dumps during lime quarrying operations. The vast majority of finds can be correlated with the rich bone accumulation of Member 3.

I have not considered biostratigraphic aspects of the Makapansgat formation here; these, together with the environmental implications of the data, will be discussed elsewhere.

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A late Proterozoic ophiolite complex at Jabal Ess in northern Saudi Arabia

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A folded and lightly-metamorphosed allochthonous thrust slice, representing a complete ophiolite succession (Penrose definition), has been mapped in the late Proterozoic shield of Saudi Arabia. This occurrence supports the suggestion that plate-tectonic motions operated during the late Proterozoic.

The late Proterozoic stratigraphy of the Arabian Shield is fundamental to the question of whether plate-tectonic motions operated in the Precambrian^{1,2}. This vast area of igneous rocks (1,200 km N–S and 600 km E–W) comprises about two-thirds plutonic rocks and one-third lightly-metamorphosed lava flows and volcanoclastics. High-grade metamorphic areas are minor. The shield is characterised by radiometric ages from 1,190 to 530 Myr^{3–5}. Within it there are many serpentinised bodies and belts⁶; some extend discontinuously for up to 700 km. The term 'ophiolite' has long been used in the description of these complexes^{7,8} and more recently some have been more convincingly identified as incomplete ophiolite complexes^{9,10} in the sense of the Penrose field meeting of 1974^{11,12}. More recently the serpentinite belts have been postulated to be ophiolitic sutures^{8,9,13,14}, formed by the closure of oceanic basins. These interpretations, amongst others, have led to a proposed mechanism of evolution of the Arabian Shield by the plate-tectonic opening and closing of sialic crust¹⁵ or by the accretion of island arcs¹⁶. However, a complete ophiolite succession in the sense of the Penrose definition has never been described in the Arabian Shield, although it has been sought for some years. The most detailed study at Jabal al Wask⁹ reported serpentinised ultramafic rocks (dunite, harzburgite, wehrlite, pyroxenite and chromitite), cumulate and high level gabbros and a metabasalt-keratophyre volcanic association. Recently an alternative

explanation for some of the serpentinite belts both in Saudi Arabia and Egypt has been proposed^{17,18}—that they are serpentinised komatiitic sills. We describe here a complete ophiolite succession from a new locality in the northern shield of Saudi Arabia (Fig. 1).

Stratigraphy of the Jabal Ess area

The three components of the mapped area are (1) a meta-sedimentary sequence; this is concordantly overlain by (2) the Jabal Ess ophiolite complex, which is in turn overlain and fault-separated from (3) an upper meta-volcanic sequence.

The stratigraphic succession of the area from bottom to top is:

(1) The meta-sedimentary sequence: south of the ophiolite complex is a sequence of lightly-metamorphosed, pale-green sediments. Strata vary from 1 to 30 m thick, with most around 2 m. The common rock type is a green shale, often with well-developed pencil cleavage. Less common are pebbly shales and conglomerates. Pebbles are well rounded and up to 20 cm in diameter. They are usually stretched, but have mainly lost their original identity due to chloritisation, with only chert and gabbro clasts recognisable. Several lenses of *mélange* up to 10 m thick and composed of gabbro and serpentinite, are concordantly contained within the meta-sedimentary sequence.

(2) The Jabal Ess ophiolite complex: this complex has a minimum exposed thickness of about 3 km, but is very condensed by folding and faulting (Fig. 1b). It has been divided into nine components, which from bottom to top are: (i) *The mélange zone*, which is up to 250 m thick, and discontinuously present along the southern limit of the ophiolite complex. The *mélange* consists of blocks of leucocratic meta-gabbro (containing meta-dolerite dykes), melanocratic meta-gabbro, pillow lava and dolerite in a sheared serpentinite host. Some blocks are up to 30 m in diameter. A lens of sheared serpentinite from 2 to 10 m

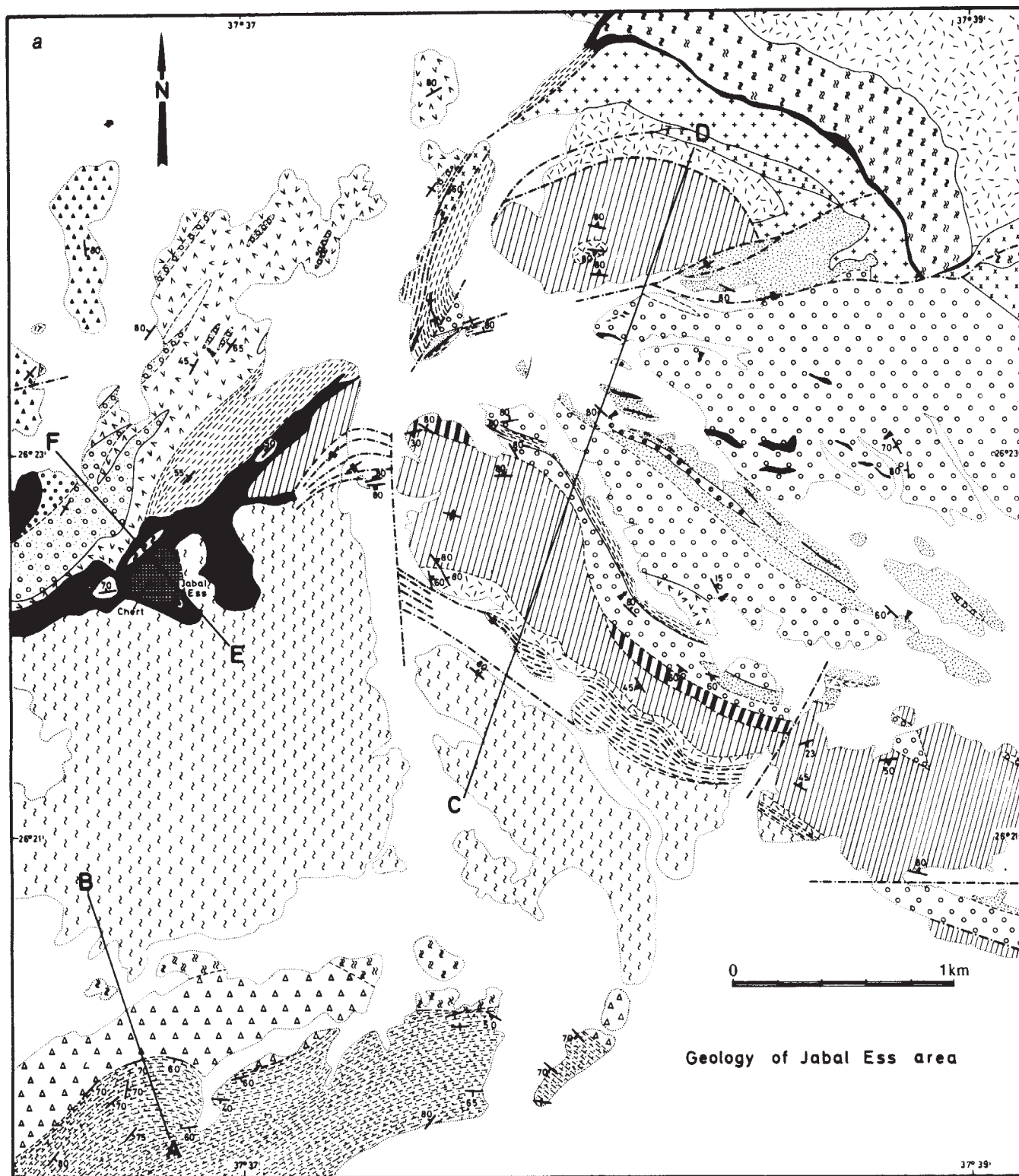
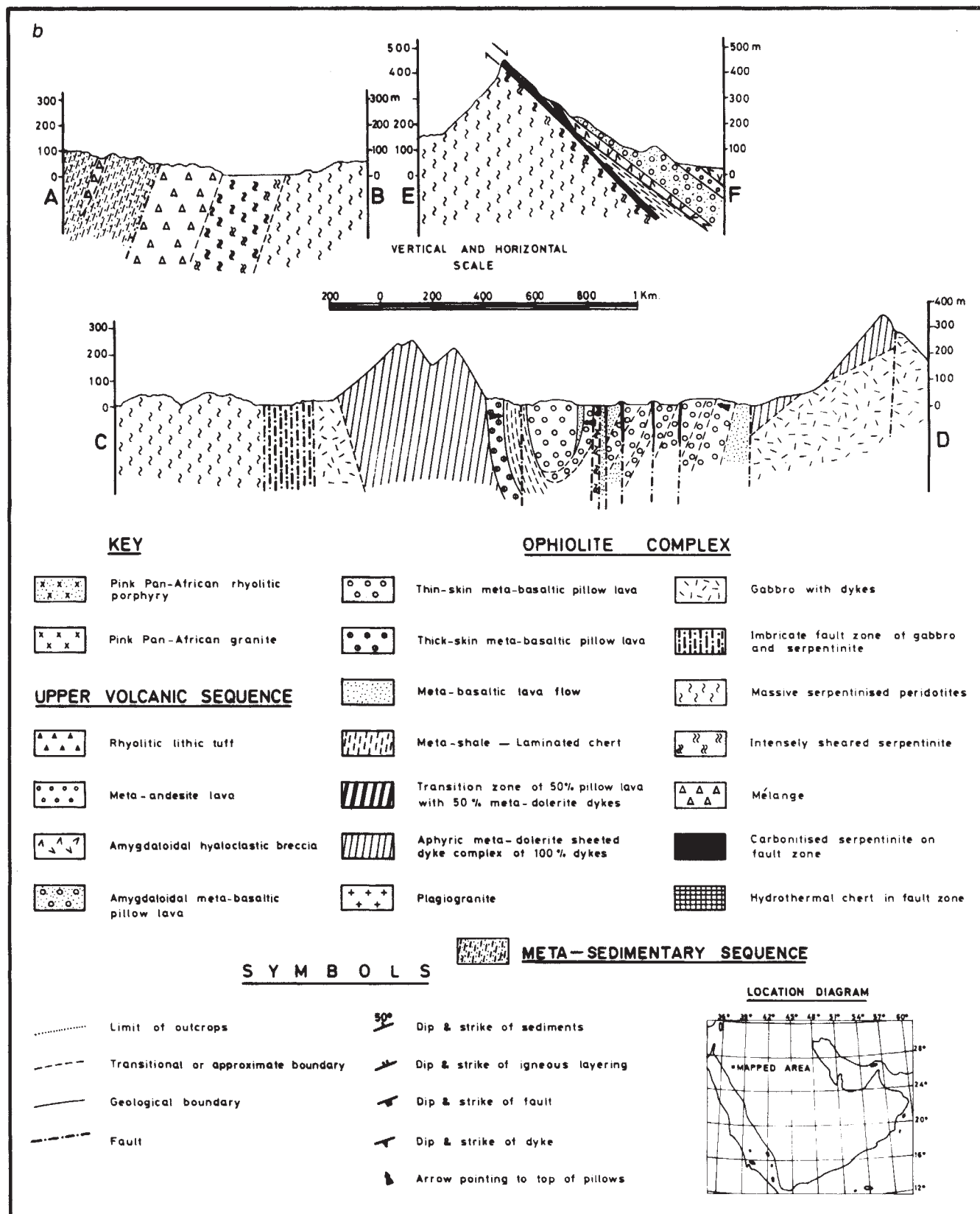


Fig. 1 a, Geological map of the Jabal Ess area, Saudi Arabia; b, (overleaf) cross-sections of the area are shown in a.

wide, which contains blocks of melanocratic meta-gabbro, occurs in direct contact with the meta-sedimentary sequence. Stratification of the meta-sediments and the contact with the mélangé are concordant but folded and overturned in the mapped area. (ii) *Serpentised metamorphic peridotite*¹²: a black, massive serpentised harzburgite with 5–20% orthopyroxene pseudomorphs shows incomplete serpentisation of olivine, usually with up to 10% relicts. Disseminated chromite is present, as well as podiform chromite lenses up to 20 cm thick. Near the contact with the mélangé, the peridotite is intensely sheared, partly carbonitised and veined by magnesite. (iii) *Serpentised cumulate peridotite*¹²: the boundary between these

and the serpentised harzburgite has not been mapped in the field. The cumulate peridotites are recognised in thin sections which show serpentised wehrlite with 50% clinopyroxene. Near the imbricate fault zone, thin (up to 30 cm) yellow-weathering layers were seen in the field which proved in thin section to be serpentised dunites. The cumulate peridotite is about 400 m thick adjacent to and south of the imbricate fault zone. The combined thicknesses of the two peridotite zones (ii and iii) is about 1.2 km. (iv) *The imbricate fault zone* is a striking and mappable unit in the field. It is up to 200 m wide and consists of a series of near-vertical fault slices from 1 to 15 m thick of light-coloured meta-gabbro and brown sheared serpentinite.



The meta-gabbro slices rarely show igneous layering and this is truncated by the faults. Usually the meta-gabbro is partly mylonitised so that blocks retaining gabbroic texture are found set in a pale, streaky, mylonitised host. Some of the thinnest fault slices (about 1 m thick) are made up entirely of mylonitised meta-gabbro and lack relict textures. This zone may have faulted out melanocratic layered gabbro as blocks of this rock are

present in the *mélange*. (v) *Layered gabbro*: a leucocratic gabbro with varying amounts of alteration shows a poorly-developed, rhythmic layering, but clear igneous lamination. Typically areas of pegmatitic-textured gabbro and aphyric meta-dolerite dykes are present. The layered gabbro is poorly represented in the mapped area but occurs within the imbricate fault zone and the sheeted dyke complex. Fault-bounded blocks of gabbro occur in

the north of the mapped area. Many outcrops show all stages of deformation of the gabbro from flaser gabbro to brecciated meta-gabbro set in a mylonitised host. (vi) *The sheeted dyke complex*: most of the complex consists entirely of meta-dolerite dykes. These have a distinctive, aphyric, doleritic texture and a green colour. The distinctive texture allows chilled margins to be recognised. Individual dykes range from 30 cm to 2 m in width. The thickness of this complex varies from 200 to 600 m. The field separation of meta-lavas from meta-dykes is easy as the lavas are much finer grained, contain sparse amygdaloids and weather brown. In the centre of the mapped area, the upper and lower boundaries of the sheeted-dyke complex are transitional over distances of up to 50 m. Along the upper contact some zones of 50% each of dykes and lavas have been mapped (Fig. 1a). Where there is <20% dykes the rocks were mapped as lavas. A striking feature of the upper half of the sheeted dyke complex is the presence of many brown and white patches and areas up to a few metres in size, where the dykes show evidence of metasomatic leaching and iron staining. The orientation of the pillow lavas adjacent to the sheeted dyke complex is near vertical or steeply inclined towards the north. The sheeted dykes immediately below the lavas are orientated at right angles to the lava stratification. Adjacent to the gabbro some of the dykes are now steeply inclined, indicating they were originally at low angles relative to the stratification of the lavas. (vii) *Meta-basalt lavas*: the sheeted dyke complex is overlain by a succession dominated by meta-basalt lavas, at least 300 m thick, but repeated by folding and faulting. Most of the lavas occur as thin-skinned pillow lavas with pale-brown, fine-grained cores and narrow, black-green, chloritised selvages, although a thick-skinned type is sparsely present (Fig. 1). This latter type is characterised by individual pillows having outer chill zones up to 8 cm thick which contain an abundance of white spherulites up to 4 mm in diameter. The cores of this type of pillow are composed of the normal meta-basalt. Almost all of the meta-lavas are aphyric, although one occurrence of plagioclase-phyric pillow lava was found. Many of the pillows have a tubular form. Some horizons of brecciated pillow lava occur and probably indicate palaeoslopes. (viii) *Shale and laminated chert*: within the meta-basalt sequence, there is a single meta-sedimentary unit up to 50 m thick. This consists of thin-bedded, fissile, khaki-coloured shale with some colour variations which indicate the stratification. Within this unit some shale horizons are siliceous and pass into thin units (<1 m thick) of laminated chert. (ix) *Plagiogranite*: a single arcuate (folded?) and fault-bounded block of plagiogranite is present in the much-faulted northern part of the mapped area (Fig. 1a). The plagiogranite is crosscut by dykes of pink Pan-African granite. The mineralogy is essentially quartz and sodic plagioclase (An_{10}). The plagioclase is turbid and there is a small amount of a chloritised mafic mineral. (3) *The upper meta-volcanic sequence* is lightly metamorphosed and consists of andesite, dacite and rhyolite lavas with sparse occurrences of pillowed meta-basalt lavas. The latter are distinct in being highly amygdaloidal in contrast to the very sparsely amygdaloidal pillow lavas of the ophiolite complex. Clastic rocks are abundant in the upper meta-volcanic sequence. Most prominent are rhyolitic lithic tuffs and multilithologic submarine slump deposits containing conspicuous rhyolite blocks up to 1.5 m in diameter. The environment of deposition evidently varied from shallow-water submarine to subaerial as some horizons have shales with mudcracks and at another welded tuffs are present.

Structure and tectonism

The Jabal Ess ophiolite complex occurs as a syncline with the gabbro-serpentinite components repeated in the north and the south of the mapped area (Fig. 1a). The abundance of pillow lava structure has been used to indicate the way up. The study (Fig. 1a and b) showed that the meta-basalt lavas are repeated by folding about the synclinal axis. The ophiolite complex is discordantly fault-separated from the overlying upper meta-

volcanic sequence. The faults are distinct because on Jabal Ess the upper meta-volcanic sequence is juxtaposed onto the serpentinised peridotite and then, towards the east, onto successively higher structural units of the ophiolite complex. The faults are carbonitised and locally silicified. This alteration along faults is best developed on Jabal Ess where up to 20 m of serpentinite have been carbonitised.

The base of the ophiolite complex is the bottom of the mélange zone which is concordant with the meta-sedimentary sequence. The latter concordantly contains lenses of mélange up to 10 m thick. The mélange zone presumably represents an original low-angle thrust fault zone which must have cut across all units of the ophiolite complex, as indicated by the content of blocks within the mélange. The Jabal Ess ophiolite complex is interpreted as an allochthonous fault slice which was emplaced by thrusting to concordantly overlie the meta-sedimentary sequence. Significantly, there are no clasts from the upper meta-volcanic sequence within the mélange. This absence suggests that the discordant set of faults, which mark the upper boundary of the ophiolite complex, postdate the basal thrust of the mélange zone.

The ophiolite complex, together with its host rocks, has been twice folded. Both folding events seem to postdate the emplacement of the allochthonous slice as well as the juxtapositioning of the ophiolite complex and upper meta-volcanic sequence. The first folding formed a tight syncline with an east-west fold axis. A later folding event resulted in more open folds about NNW axes, so that the ophiolite complex has a sigmoidal form. The combination of the two folding events has resulted in near-vertical dips for most of the mapped area and the basal mélange zone of the ophiolite complex is now overturned. Only the main faults are shown on the map (Fig. 1a).

Conclusions

We have confirmed that Proterozoic ophiolite complexes in the sense of the Penrose meeting do exist in the Saudi Arabian Shield. Metamorphism has not obliterated the original rock features in this occurrence, which can be used as a type locality for Arabian Proterozoic ophiolites. The area is readily accessible by road or light aircraft. The recognition of such units as the aphyric sheeted dyke complex will identify similar rocks in other more tectonised areas of the shield.

The presence of an allochthonous thrust slice of Proterozoic ophiolite within the shield is a strong indication that plate-tectonic motions operated during the late Proterozoic. This conclusion strongly supports an accretionary type of model for the crustal evolution of the large igneous terrain of the Arabian Shield.

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Modulation of the two promoters of the galactose operon of *Escherichia coli*

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The gal operon of Escherichia coli is controlled by two independent promoters—one is activated and the other inhibited by cyclic AMP and cyclic AMP receptor protein. The two promoters are modulated, however, by the same operator locus and repressor protein.

THE galactose (*gal*) operon of *Escherichia coli* consists of three structural genes, *K*, *T* and *E*, with the promoter-operator (*P-O*) located at the *E* end of the transcriptional unit¹ (Fig. 1). In wild-type *E. coli* cells, expression of the *gal* genes is inducible by D-galactose or D-fucose². The inducer prevents a specific repressor protein, the product of the unlinked *galR* gene, from binding to the *gal* operator³. The operator has been defined genetically by the isolation of *cis* dominant mutations, which map to the right of the *E* gene and make *gal* expression constitutive.

It has been shown both *in vivo* and *in vitro* that the *gal* operon is controlled by two promoters: *P1* and *P2*⁴. The *P1* promoter requires cyclic AMP and cyclic AMP receptor protein (CRP) as positive control elements for its activity; *P1* is normally responsible for *gal* expression in wild-type cells. The *P2* promoter does not need the cyclic nucleotide and the receptor protein and is in fact inhibited by the presence of the two regulatory elements. The *in vitro* startpoints of transcription from the two *gal* promoters, *S1* and *S2*, are also different and separated by five nucleotides (Fig. 1)⁴. In normal conditions of *in vitro* transcription, that is, in the presence of excess nucleoside triphosphate precursors, transcription from *P2*, starting at *S2*, largely terminates after completing a trinucleotide leader RNA. The reason for this premature termination is not clear and will be touched upon later.

We report in this article that the synthesis of *gal* enzymes under *P2* control, like that under *P1*, is also inducible and is under the negative control of the *galR* gene product. The same DNA segment in *gal*, defined by the *O*^c mutations and by *in vitro* *gal* repressor binding experiments, is required for inhibition of both *P1* and *P2* activities. The accompanying article⁵ shows that the location of the *gal* operator with respect to the *gal* promoters differs markedly from other known promoter-operator structures (Fig. 1).

Induction of *gal* operon

In wild-type *E. coli* cells, under conditions of high cyclic AMP levels, the *gal* enzymes are made almost entirely from the *P1* promoter, whereas in cells deficient in cyclic AMP or CRP, the *P2* promoter is responsible for the expression of the operon. Thus, the regulatory features of the two promoters can be studied separately simply by measuring the *gal* enzymes made in *cya*⁺*crp*⁺ and *cya*⁻ (or *crp*⁻) strains, respectively. Evidence that both promoters are not on together *in vivo* in the presence of inducer and cyclic AMP-CRP is provided by the finding that the *P1*⁻ mutation is Gal⁻ in *cya*⁺*crp*⁺ strains. Table 1 compares the effect of the *gal* inducer D-galactose on the two promoter activities as measured by galactokinase synthesis, the product of the *galK* cistron, in the two strains SA500 (wild type) and SA1039 (*cya*⁻). Clearly, both the promoters are inducible. This is the first experimental evidence that *galP2* is inducible; previous measurements of *gal* induction and repression reflected *gal* expression controlled by *P1*, the cyclic AMP dependent

promoter. It should be noted that the actual levels of galactokinase, the last gene product, made from a promoter need not reflect the strength of that promoter. It is possible that the two promoters might show different degrees of natural polarity (S. A. and S. Gottesman, in preparation).

However, similar galactokinase induction ratios (about 15-fold) with the two promoters and the fact that both *P1* and *P2* activities can be induced by the same inducers, D-galactose (Table 1) and D-fucose (data not shown), suggest that *P2* is controlled by the same repressor molecule that controls *P1*, that is, the *galR* gene product. This was corroborated as follows. The *cya*⁻ mutation of SA1039 was introduced into a set of strains, isogenic with wild type strain (SA500), which harbour a series of *galR* mutations. Comparison of galactokinase levels between the corresponding *galR*⁻ *cya*⁺ and *galR*⁻ *cya*⁻ strains showed constitutive synthesis in each case, including a *galR* deletion strain (Table 1). The *galR*⁻ mutations have previously been shown to be recessive to the *galR*⁺ allele⁶.

Identical negative control of both the promoters by the same *gal* repressor was confirmed by studying the effect of a *galR*^S mutation on *P1* and *P2*. A *galR*^S mutation has been shown to produce a non-inducible phenotype in a wild-type background and is dominant over *galR*⁺ (refs 1, 7). The phenotype is the result of the altered repressor protein, which fails to interact with the inducer molecule. The *galR*₇₈^S mutation retains its phenotype in a *cya*⁻ background, that is, galactokinase synthesis remains at the low basal levels both in *cya*⁺ *galR*^S (SA1796) and *cya*⁻ *galR*^S (SA1800) strains in the presence or absence of D-galactose (Table 1).

However, it is conceivable that the non-inducible Gal⁻ phenotype of the *cya*⁻ *galR*^S strain is the result of the lack of an effective permease for D-galactose transport into the cell. This

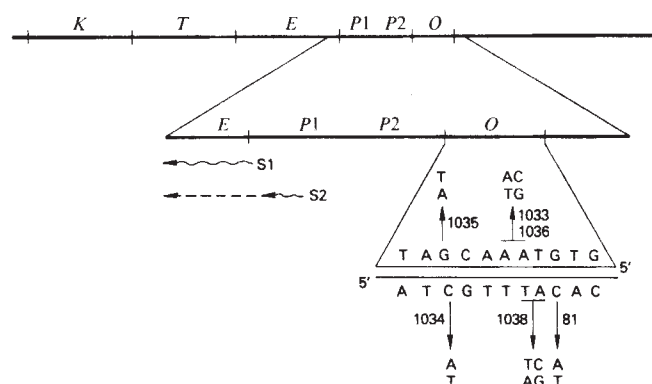


Fig. 1 Map of the *gal* operon, showing the operator mutations. *K*, *T* and *E* are the structural genes¹. *P1* and *P2* are the two promoters, whose positions relative to each other are not known. There may be considerable overlap between the two loci, except that *P1* mutations do not affect the *P2* activity⁴. *S1* and *S2* represent startpoints of transcription for their corresponding promoters *P1* and *P2*. As shown, the *S2* transcript frequently terminates after synthesising a trinucleotide. The operator segment shows the nucleotide sequence together with the base pair changes of the six *O*^c mutations⁵. The map is not drawn to scale.

Table 1 Effect of repressor on *gal* promoters

Strains		Active promoter	Levels of galactokinase*	
No.	Genotype		-Galactose	+Galactose
SA500	Wild type	<i>P1</i>	0.9	15.7
SA1039	<i>cya</i> ⁻	<i>P2</i>	0.6	9.0
SA1857	<i>galR</i> ₃ ⁻	<i>P1</i>	8.5	7.8
SA1858	<i>galR</i> ₃ ⁻ <i>cya</i> ⁻	<i>P2</i>	14.4	11.0
SA1859	<i>galR</i> _{B78} ⁻	<i>P1</i>	8.3	7.3
SA1860	<i>galR</i> _{B78} <i>cya</i> ⁻	<i>P2</i>	10.8	7.4
SA1260	<i>galR</i> _Δ	<i>P1</i>	11.8	12.5
SA1775	<i>galR</i> _Δ <i>cya</i> ⁻	<i>P2</i>	20.6	12.0
SA1796	<i>galR</i> ₇₈ ^S	<i>P1</i>	0.3	0.4
SA1800	<i>galR</i> ₇₈ ^S <i>cya</i> ⁻	<i>P2</i>	1.4	0.9
SA1953	<i>galR</i> ₇₈ ^S <i>cya</i> ⁻ <i>lacP</i> _{uv5}	<i>P2</i>	0.7	0.7

Galactokinase was assayed in lysed cells after 2 h of log phase growth in minimal M56 media containing 0.3% D-fructose as carbon source, 0.1% casaminoacids and 0.3% D-galactose as inducer when indicated. Cells were lysed by 0.1 M Tris-HCl, pH 7.9, 0.1 M EDTA and 0.03 M dithiothreitol plus a drop of toluene at 37 °C for 15 min. The wild-type strain, SA500, is *F*⁻ *str*^R *his*⁻ *rel*⁻. All others were derived from SA500. The *galR*_Δ also deletes the adjacent *lysA* region¹⁴. The *galR*₇₈^S (ref. 1) mutation was introduced into the *galR*_Δ strain by phage P1 selecting for *lys*⁺ and screening for *galR*₇₈^S. The galactokinase assay procedure is from Wilson and Hogness¹⁵.

*Units: nmol galactose-1-phosphate generated per min by the number of cells equivalent to A₅₄₀ of 1.0.

objection was ruled out as follows. Lactose permease, the product of the *lacY* gene, efficiently transports D-galactose⁶. Active *lac* enzymes can be synthesised in a *cya*⁻ strain when the *lac* operon carries the *uv5* promoter mutation⁸. Accordingly, the strain *galR*₇₈^S *cya*⁻ was made into *galR*₇₈^S *cya*⁻ *lacP*_{uv5} to ensure an effective D-galactose transport by selecting for Lac⁺ transductant after infection of a bacteriophage P1 lysate made on a *cya*⁻ *lacP*_{uv5} host. Table 1 shows that similar to *galR*₇₈^S *cya*⁻ (SA1800) parent, the triple mutant *galR*₇₈^S *cya*⁻ *lacP*_{uv5} (SA1953) is still non-inducible for galactokinase. Taken together, the results with the *galR* mutants convincingly show that the same repressor molecule controls *gal* expression negatively both in the absence and presence of cyclic AMP, that is, both from the *P1* and *P2* promoters.

The *gal* operator

Using a strain diploid for the *galR*⁺ gene, that is, *galR*⁺/*galR*⁺, several *E. coli* mutants constitutive for the *gal* operon were isolated after UV mutagenesis and then enrichment and selection for growth on galactose minimal media in the presence of an anti-inducer of the operon, methyl-β-D-thiogalactoside as previously described^{3,6,10}. The *galR*⁺ diploid was made by lysogenising wild type strain SA500 with a λ*galR*⁺ transducing phage isolated before⁹. Many such mutants carry *cis*-dominant, *gal*-linked constitutive mutations (S.A., unpublished results). These mutations define the *gal* operator (the site of repressor action). Six such independently isolated operator constitutive mutations (*O*^c), including one that was isolated previously in a *galR*⁺ haploid strain³, were further characterised as follows:

(1) All six mutations, listed in Table 2, cause very high levels of galactokinase synthesis in the absence of any inducer. The same high levels of galactokinase were made even in the *cya*⁻ derivatives of the *O*^c mutants. If the *gal* operon in the *O*^c mutants, as in the *O*⁺ parent, is transcribed from the *P2* promoter in the absence of cyclic AMP, these results suggest that the same mutations are responsible for making the *gal* expression constitutive both from *P1* and from *P2*.

(2) The levels of galactokinase in four of the six *O*^c mutants, *O*₈₁^c, *O*₁₀₃₈^c, *O*₁₀₃₃^c and *O*₁₀₃₆^c, cannot be further increased by the addition of D-galactose to the growth media under conditions of both *P1* and *P2* activities. This contrasts the *galO*^c mutants with

O^c mutants in other operons, which usually can be further induced. The behaviour of the *galO*^c mutants may be attributed perhaps to the demand of the selection procedure used.

(3) The levels of galactokinase in the same four *O*^c mutants are significantly higher than that in the fully induced *O*⁺ cells. This is true, both in *cya*⁺ and *cya*⁻ backgrounds. The *galO*^c mutants are, in these respects as well, unique. We have considered three possibilities to explain the hyperactivity of the *galO*^c mutants: (i) The *galO*^c mutations have in fact created new cyclic AMP-dependent promoters, transcription from which is not repressible by the *gal* repressor. This may also explain property (2) described above. (ii) The two *gal* promoters and the *gal* operator sequences overlap such that the *O*^c mutations increase the efficiency of the promoters. (iii) The natural polarity observed in the *gal* operon, that is, synthesis of reduced amount of product from the promoter-distal *K* gene (S. A. and S. Gottesman, in preparation), is eliminated by the *O*^c mutations in an unknown way. Elimination of natural polarity would increase the levels of galactokinase. *gal* DNA templates, carrying either *O*⁺ or one of the six *O*^c alleles, behave identically in a purified transcription system, that is, they need cyclic AMP for transcription from the *P1* promoter but not from *P2* (refs 5, 11). The latter was always inhibited by the presence of cyclic AMP and CRP. These results do not rule out, but argue against possibility (i). We are now studying possibilities (ii) and (iii).

(4) The accompanying article⁵ shows the base-pair alterations of the six *O*^c mutations in the *gal* control region (indicated in Fig. 1). All six mutations cluster in a small region (spanning seven bases). This segment is located about 60 nucleotides upstream from the start site of the S1 transcript. The short length of the *galO* segment and the fact that two of the mutations, *O*₁₀₃₃^c and *O*₁₀₃₆^c, show identical base-pair changes and two others (*O*₁₀₃₄^c and *O*₁₀₃₅^c) affect the same base pair suggest uniqueness of the operator structure. [Note that half of the mutations show simultaneous change of two adjacent base pairs. This may be attributed to the nature of the mutagen used (UV light) to induce the mutations. UV light has been shown to cause similar tandem base-pair changes in other *E. coli* genes¹².]

Discussion

The *gal* operon of *E. coli* is unique in that the same structural genes are controlled by two natural promoters. *P1* and *P2* meet the additional requirements of galactose enzymes for anabolic reactions under extreme physiological conditions—excess or deficiency of cyclic AMP. The results discussed above show that both promoters are subject to repression. The same regulatory elements, *gal* repressor, D-galactose and the *gal* operator, control the *gal* expression from both *P1* and *P2*. A contrasting feature of the two promoters is that one (*P1*) is activated and the other (*P2*) inhibited by cyclic AMP and its receptor protein. For *P2*, cyclic AMP and CRP together are not co-repressors acting

Table 2 Effect of operator mutations on the *gal* promoters

Operator allele	Levels of galactokinase in			
	<i>cya</i> ⁺	<i>cya</i> ⁻	<i>cya</i> ⁺	<i>cya</i> ⁻
<i>O</i> ⁺	-I	+I	-I	+I
<i>O</i> ₈₁ ^c	1.0	18.0	0.7	8.1
<i>O</i> ₁₀₃₈ ^c	31.1	36.1	30.5	29.6
<i>O</i> ₁₀₃₃ ^c	32.0	34.3	34.0	32.0
<i>O</i> ₁₀₃₆ ^c	34.0	33.4	36.0	31.3
<i>O</i> ₁₀₃₄ ^c	33.1	34.2	34.6	32.0
<i>O</i> ₁₀₃₅ ^c	9.2	14.9	26.0	29.2
	31.2	31.3	15.0	29.4

The *O*⁺ strain is SA500 described in Table 1. The strains are isogenic with SA500. The *cya*⁻ mutation was transduced into the *O*^c mutants with phage P1 by selecting for a *val*^R marker and screening for Lac⁻ phenotype. The *val*^R marker is 40% co-transducible with the *cya*⁻ allele (A. Das, unpublished results). The other details are also described in Table 1. -I and +I indicate the absence and presence of D-galactose (inducer) during cell growth.

in concert with the *gal* repressor, because *crp*⁻ mutants do not make the *gal* enzymes constitutively. The molecular mechanisms of action of CRP have been explored and are discussed in the accompanying article⁵.

We have shown above that the *gal* repressor represses *gal* transcription from both *P*₁ and *P*₂ by acting at the same operator site, as defined by *O*^c mutations. *In vitro gal* transcription from the *P*₁ promoter in a purified system in the presence of cyclic AMP and CRP is inhibited by the addition of the *gal* repressor¹¹. As expected, the *gal* repressor failed to show repression when the template DNA carried any one of the six *O*^c mutations discussed above^{5,11}. In repressing the *P*₁ promoter, *gal* repressor acts in a manner competitive with RNA polymerase plus CRP¹³. However, the mechanism of action of *gal* repressor may be different from simple competition, because the operator is located at an unusual site—well upstream from the RNA polymerase or CRP binding site. The synthesis of the

short S2 transcripts made from the *galOP* restriction fragment in the absence of cyclic AMP and CRP (that is, under the control of the *P*₂ promoter), however, is not inhibited by the *gal* repressor⁵. The *gal* repressor may fail to repress S2 synthesis in the purified transcription system for at least two possible reasons. (1) Lack of an additional element besides the *gal* repressor is needed for repression of *P*₂ activity. And (2), *gal* transcription from the *P*₂ promoter is not controlled by inhibition of initiation, but by elongation of the short S2 transcript. The *gal* repressor might act by inhibiting the elongation of S2 RNA.

We are currently investigating these possibilities in order to explain the above discrepancy between *in vivo* and *in vitro* control of the *gal* operon.

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Unusual location and function of the operator in the *Escherichia coli* galactose operon

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The operator of the gal operon is located about 60 base pairs preceding the startpoints of the transcription of the two gal promoters. This location contrasts with the location of the operator in other phage or bacterial operons where the repressor binds more closely to the respective transcription initiation sites. Models explaining how the repressor-operator interactions may control the two gal promoters are presented.

OUR previous studies have shown that two overlapping promoters control the expression of the galactose operon of *Escherichia coli*. *In vitro* transcription experiments of a DNA fragment containing the *gal* operator-promoter region demonstrated that cyclic AMP and its receptor protein (CRP) were required for the activity of one promoter (*P*₁), but inhibited transcription at the other promoter (*P*₂)¹. The transcription initiation site for *P*₂ precedes the startpoint for *P*₁ by five base pairs in the DNA sequence. We presented arguments which strongly suggest that these two promoters are active *in vivo* under different physiological conditions¹.

In addition to this dual cyclic AMP-CRP regulation, the *gal* operon is also controlled by the *gal* repressor, the product of the *galR* gene which is unlinked to the *gal* operon^{2,3}. Earlier studies had shown that the *gal* repressor binds specifically and with a

very high affinity to *gal* DNA⁴ and blocks *gal* transcription *in vitro* with wild-type *gal* DNA but not with *gal* DNA with operator-constitutive mutations⁵. We report here on the effect of the *gal* repressor at each of the two *gal* promoters, and on the unusual location of the repressor binding site.

By sequencing five *gal* operator mutations we show that the repressor binds about 60–65 base pairs upstream from the startpoint for cyclic AMP-CRP dependent transcription. This contrasts with the location of the operator in the *lac*⁶, *trp*⁷ and *lambda* operons. In the latter systems the repressor binds much more closely to the respective initiation sites for transcription and probably directly prevents the stable binding of RNA polymerase to the promoters. The location of the *gal* operator suggests that in *gal* the repressor prevents transcription by a different mechanism.

We also show that in our purified *in vitro* transcription system the *gal* repressor inhibits transcription from *P*₁; it does not block initiation of transcription at *P*₂ in the absence of cyclic AMP-CRP and does not prevent the cyclic AMP-CRP dependent inhibition of *P*₂. In contrast, *in vivo* results presented in the accompanying article⁹ indicate that both *P*₁ and *P*₂ are under repressor control. Possible explanations for this discrepancy as well as models for the mechanism of action of the *gal* repressor will be discussed.

Sequence changes in *galO*^c mutants

We have examined five *gal*-linked *cis*-dominant mutants which exhibit a high constitutive rate of synthesis of the *gal* enzymes. Several lines of evidence indicate that these *gal* mutations define a site to which the *gal* repressor binds (see also accompanying article⁹). (1) The mutants were selected as capable of growing in

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Table 1 The presence of a plasmid harbouring a wild-type or mutant *gal* operator affects expression of the chromosomal *gal* genes

Strain	Genotype of <i>gal</i> operator in plasmid	Galactokinase (units)	
		-D-fucose	+D-fucose
MR94	I	2.8	24.8
MR94/pBdC1	O ⁺	18.1	36.9
MR94/pBdC6	O ^c	4.7	20.5

Plasmid pBdC1 is derived from pBR322 in which the 30 base pair fragment between the unique *Eco*RI and *Hind*III sites of pBR322 has been replaced by an *Eco*RI-*Hind*III fragment derived from λ gal8 DNA²¹. The inserted fragment has a size of about 1,000 base pairs and contains the operator-promoter region of the *gal* operon and about 450 base pairs of the first *gal* cistron (B.deC., in preparation). pBdC1 contains a wild-type *gal* operator whereas pBdC6 contains the O₈₁^c *gal* mutation (see Fig. 4a). Medium for growth of cells and assay conditions for galactokinase were as described previously¹. MR94 is a *recA* derivation of MR93 (collection of E. Singer) constructed by C. B. Bruni.

a minimal galactose medium in the presence of the anti-inducer of the *gal* operon, methyl- β -D-thiogalactoside (TMG), a property which is also common to *galR* mutants¹⁰. Presumably, in both types of mutants no stable *gal* repressor-operator complex is formed, thus expression of the operon is constitutive and these cells can grow in a minimal salts medium containing galactose plus TMG. (2) Earlier *in vivo* transcription studies with λ *gal* DNA showed that the *gal* repressor blocks cyclic AMP-CRP dependent *gal* mRNA synthesis (as measured by DNA-RNA hybridisation) with wild-type *gal* DNA but not with the DNA from a *galO^c* mutant⁵. (3) We have constructed a plasmid (derived from pBR322) which contains the *gal* operator-promoter region and the promoter proximal third of the *galE* cistron (B.deC., manuscript in preparation). Wild-type cells transformed with this plasmid are also capable of growing in minimal galactose plus TMG. The 30 or more copies of *gal* operator titrate the *gal* repressor and cause the constitutive 'escape' expression of the chromosomal *gal* genes. If a *galO^c* mutation is introduced in the plasmid, the cells are incapable of growing on minimal galactose plus TMG and exhibit a normal induction pattern of *gal* enzyme synthesis (Table 1). We can assume that, unlike the parent plasmid, the plasmid with a *galO^c* mutation is unable to titrate the cellular *gal* repressor. Hence, the mutation marks the repressor binding site in the *gal* regulatory region.

We have previously established a restriction map for the regulatory DNA segment preceding the first *gal* structural gene¹¹. Figure 1 shows the restriction sites of interest. Endonuclease *Hinf* cleaves wild-type *gal* DNA 60/59 base pairs (on the I strand) preceding S1, the startpoint for cyclic AMP-CRP dependent transcription. With two of the mutants (O₁₀₃₄^c and O₁₀₃₅^c) the DNA is not cleaved by *Hinf* at this site (data not shown), suggesting that these mutations may lie within the recognition site for *Hinf* endonuclease.

The base changes for each of five mutants were determined. The sequence data are detailed below. The 35 base pair DNA fragment 1 (see Fig. 1) from mutant O₈₁^c was labelled selectively at its *Hinf* 5' end and digested by snake venom phosphodiesterase. The partial exonuclease digestion products of the mutant fragment and of a similarly treated wild-type DNA fragment were mixed and fractionated by two-dimensional fingerprint analysis¹⁹. Figure 2a compares the mobility shifts of the fractionation products of the mutant fragment with those of the wild-type fragment. The patterns deviate at the site of the mutation indicating that the C residue (-66) in the wild-type sequence¹⁹ is replaced by a T residue in the mutant. Immediately following the mutated residue the succession of mobility shifts runs parallel in the mutant and in the wild-type pattern. Hence no other base change occurs adjacent to the first mutation. That the new shift in the mutant is caused by a T residue and not by a G is indicated by analysis of its mobility shift and the mobility

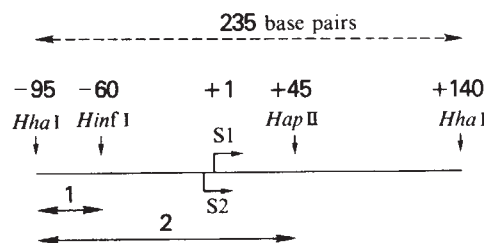


Fig. 1 Restriction map of the *gal* operator-promoter region. S1 is the startpoint for cyclic AMP-CRP dependent *gal* transcription, S2 is the startsite for cyclic AMP-CRP independent *gal* transcription. 1 and 2 refer to restriction fragments used for sequencing studies. The DNA sequence of the *gal* operator-promoter segment has been determined¹⁹.

shifts produced by the addition to known T and G residues in the wild-type sequence. Furthermore, when the same fragment I of mutant O₈₁^c, also uniquely labelled at its *Hinf* 5' end, was treated with dimethylsulphate (DMS) according to the procedure of Maxam and Gilbert¹⁰ and the fractionation products analysed by gel electrophoresis, it was clear that no new G residue appeared at position -66 (data not shown).

A similar analysis was applied to the DNA of mutant O₁₀₃₃^c. The two-dimensional fractionation of the partial nuclease degradation products of fragment I shows that two adjacent T residues in the wild-type sequence (-64 and -63) have been replaced by an A residue (-63) and a C residue (-64) (Fig. 2b). A DMS analysis on the same mutant fragment, labelled at the

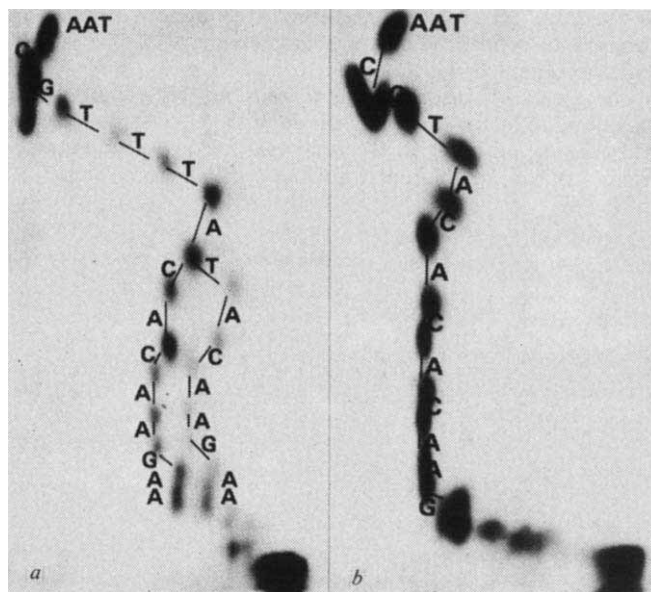


Fig. 2 Partial venom exonuclease digests of fragment 1 (of Fig. 1) terminally labelled at its *Hinf* 5' end from wild type and from the mutants O₁₂₆^c and O₁₀₃₃^c. A fragment obtained by *Hinf* cleavage, which contains DNA to the left of the *Hinf* site in Fig. 1, was prepared as in ref. 11. This fragment was purified from λ gal8 DNA²¹ (wild type) or its isogenic derivatives containing the O₁₂₆^c or the O₁₀₃₃^c mutation. The fragments were dephosphorylated with bacterial alkaline phosphatase, 5'-end labelled with [α -³²P]ATP using T4 polynucleotide kinase¹⁹ and digested with endonuclease *Hha*I. The products were resolved on a 10% polyacrylamide gel, and fragment 1 of Fig. 1 was eluted from the gel as described elsewhere¹¹. Wild-type or mutant fragment 1 (individually or in combination) was partially digested with venom exonuclease in the presence of trace amounts of DNase I and fractionated by electrophoresis on Cellologel in 8M urea at pH 3.5 followed by homochromatography¹⁹. a, O₁₂₆^c DNA and wild-type DNA; b, O₁₀₃₃^c DNA.

Hinf 5' end, confirmed the presence of an A change at -63 (data not shown).

As indicated earlier, the DNAs of mutants O_{1034}^c and O_{1035}^c lack the *Hinf* cleavage site at -60/-59 and hence were not amenable to the same partial exonuclease analysis as the preceding mutants. Fragment 2 (*Hha*-1/*Hap*-1) was end-labelled at the 5' *Hha*-end for each of these two mutants and sequenced according to Maxam and Gilbert¹². Figure 3a compares the fractionation products resulting from the DMS and hydrazine reactions of wild-type DNA and O_{1034}^c DNA. A single base substitution, A for G, occurs in the mutant DNA at -60. The base pair which is mutated in O_{1034}^c thus lies within the *Hinf* site.

In mutant O_{1035}^c the same G/C base pair (-60) is changed to a T/A base pair. Figure 3b shows the products of chemical fractionation of the *Hha*-1/*Hap*-1 fragment (fragment 2 of Fig. 1) for this mutant. Comparison with the wild-type sequence in Fig. 4 indicates the position of the base change.

A similar analysis was performed with the same *Hha*-1/*Hap*-1 DNA fragment of mutant O_{1038}^c (Fig. 3c). In this mutant two adjacent base pairs G/C and T/A have replaced the two adjacent A/T base pairs at -65 and -64.

Figure 4a summarises the results of the sequence analysis of the five mutants. The mutations which we examined are all clustered in a small segment between -66 and -60.

Effect of *gal* repressor on *in vitro* transcription

In vivo experiments⁹ indicate that the interactions between the *gal* repressor and the *gal* operator repress both *P1* and *P2*. We have also studied the effect of *gal* repressor on initiation of transcription *in vitro* at each of the two startpoints. When the 235 base pair *Hha*I fragment (see Fig. 1) is used as template and the transcription reactions are conducted at low nucleoside triphosphate concentrations, a characteristic pattern of small RNAs is obtained which results from the pausing of RNA polymerase early after initiation of transcription¹. These small RNAs can be analysed by polyacrylamide gel electrophoresis. Transcription initiating at S1 generates a diagnostic pattern, which is strikingly different from the pattern produced when

Table 2 Efficiency of transcription initiation at *P1* and *P2*

	Specific activity per UMP residue	
	in 3-nucleotide-long oligonucleotide	in 9-nucleotide-long oligonucleotide
+cyclic AMP-CRP	—	1,100
-cyclic AMP-CRP	5,700	—

Comparison of the efficiency of initiation at S1 and S2. The 235 base pair *Hha*I fragment of Fig. 1 was transcribed as described in the legend of Fig. 5, both in the absence and the presence of cyclic AMP; the RNA was then fractionated on an 18% polyacrylamide gel. From each lane the major RNA band (corresponding to a trinucleotide in the minus cyclic AMP reaction and to a nonanucleotide in the plus cyclic AMP reaction) was located by autoradiography, excised from the gel and counted. The trinucleotide and the nonanucleotide each contain two UMP residues.

transcription starts from S2. In the presence of cyclic AMP-CRP the majority of the *gal* transcripts pause at several points within the first nine bases of the 5' end of the cyclic AMP-CRP dependent *gal* mRNA^{1,22}. In the absence of cyclic AMP-CRP, transcription from S1 is completely inhibited and instead is initiated five bases upstream and pauses within a different six-base sequence. The nucleotide sequence of the various short transcripts was previously established¹. Figure 5 shows that in the absence of cyclic AMP and CRP the *gal* repressor does not cause inhibition of transcription (lane 2). Hence in the conditions of our *in vitro* reaction, the *gal* repressor is unable to prevent initiation of transcription from S2. This conclusion is also valid when transcription reactions are conducted at high nucleoside triphosphate concentrations (data not shown). We have in fact observed a small but consistent increase in S2 RNA synthesis by *gal* repressor preparations (Fig. 5 lane 2). This increase is reversible by D-fucose.

In the presence of cyclic AMP and CRP, however, the *gal* repressor blocks S1 transcription and this repression is overcome by the *gal* operon inducer D-fucose. Although the *gal* operator is located in a segment which shows striking sequence

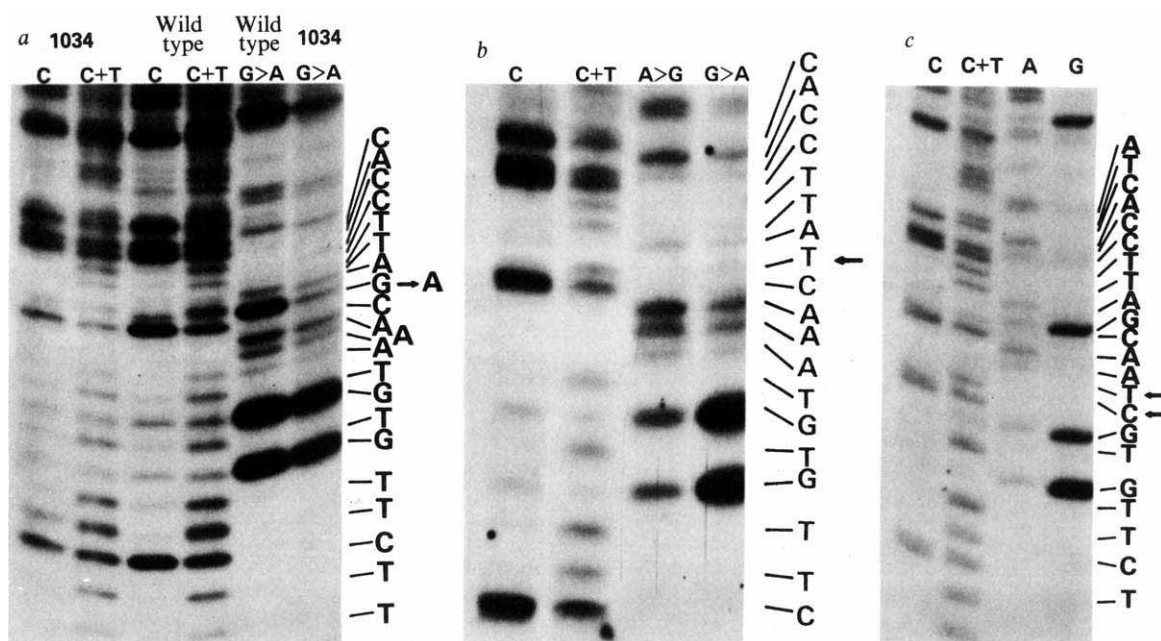


Fig. 3 Autoradiographs of DNA sequencing gels. Fragment 2 of Fig. 1 from either wild-type DNA or from the mutant DNAs O_{1034}^c , O_{1035}^c or O_{1038}^c was ³²P-labelled at its 5' *Hha* end and was subjected to base-specific chemical degradation according to Maxam and Gilbert¹⁰. The reaction products were fractionated on 20% polyacrylamide gels in 7M urea. The arrows indicate the site of the mutations. a, Comparison of wild-type DNA and O_{1034}^c DNA; b, O_{1035}^c DNA; c, O_{1038}^c DNA.

similarities and lies at the same distance from the initiation site as the CRP binding site in the *lac* operon, addition of repressor does not prevent the cyclic AMP–CRP dependent inhibition of transcription from S2 (Fig. 5, compare lanes 4 and 5). Our experiments thus clearly show that if both the *gal* repressor and cyclic AMP–CRP are present the two *gal* promoters are strongly inhibited.

Transcription of *gal* operator constitutive mutants

The isolation of mutants which do not bind cyclic AMP–CRP¹³ as well as direct chemical protection experiments¹⁴ have defined the CRP binding site in the *lac* operon (~–70 to –50). Examination of a similarly located segment in *gal* reveals important similarities both in sequence and in symmetry (see Fig. 4b). Since all *galO^c* mutations which we examined lie within this region (–66 to –60), we determined whether these mutations affected the cyclic AMP–CRP regulation of one or the other *gal* promoter. With every *galO^c* mutant transcription from S1 is still completely dependent on cyclic AMP–CRP, and transcription at S2 only initiates in the absence of cyclic AMP–CRP (data not shown). One *gal* mutant (*galO₈₁^c*) was particularly interesting to study since the same base change G/C to A/T at –66 occurs in a *lac* mutant¹³ at exactly the same location with respect to the startpoint of transcription in *lac* and S1 in *gal*. These mutations in *lac* and *gal* also occupy the same location within a similar symmetrical sequence (see Fig. 4) that seems to be the CRP binding site in *lac*. In *lac* the mutation causes severe reduction of promoter activity. In *gal* the same mutation results in the constitutive expression of the *gal* operon *in vivo* both in the presence or absence of CRP or cyclic AMP. *In vitro* the CRP concentration dependence and the cyclic AMP concentration dependence for both *P1* activation and *P2* repression are identical whether *galO₈₁^c* DNA or wild-type DNA are used as a template. Figure 6 shows the effect of cyclic AMP concentration of *P1* and *P2* transcription for both types of DNA. Hence the mutation does not affect cyclic AMP–CRP function in *gal* in contrast to its profound effect on CRP action in *lac*.

Initiation at *P2* is more efficient than initiation at *P1*

In an effort to characterise the *P2* promoter further, we compared the efficiency of initiation of transcription at S1 and S2. The characteristic small RNAs corresponding to each promoter as well as the larger RNA, which extends to the end of the fragment, were eluted from the polyacrylamide gel. The RNA sequence of each of the small RNAs is known, and their relative molar yields were determined from the radioactivity of the eluted material ([α -³²P]UTP was used in these experiments). The results shown in Table 2 indicate that in our experimental conditions transcription initiates at S2 about five times more efficiently than at S1.

Transcription of *P1* and *P2* at high nucleoside triphosphate concentrations

We also compared the pattern of transcription from *P1* and *P2* at low and at high nucleoside triphosphate (NTP) concentrations. If high (150–200 μ M) instead of low (10 μ M) NTP concentrations are used for *in vitro gal* RNA synthesis from the *Hha*-230 base pair fragment, stuttering is no longer observed for cyclic AMP–CRP dependent transcription from S1: all transcripts are of large size and extend to the end of the fragment. In the absence of cyclic AMP–CRP using high NTP concentrations, however, a considerable proportion of transcripts originating at S2 still terminates within the sequence of the first 6 or 7 bases (Fig. 7). Hence the termination of transcription from S2 is not just a result of the low NTP concentrations, unlike the pausing observed for S1 transcripts, but may represent an additional regulatory mechanism. Figure 7 also shows that in these conditions, when glycerol was present, less termination and more read-through to the end of the fragment are observed. Glycerol was shown previously to stimulate *gal* transcription from S2 in the absence of cyclic AMP–CRP^{1,15}.

Functional interactions between cyclic AMP–CRP, *gal* repressor and *gal* DNA

The DNA sequencing results presented in this article mark the location of the *gal* operator. The base changes produced by five *O^c* mutations which we examined all lie between residues –60 and –66. We have summarised the evidence which indicates that these mutations alter the binding of *gal* repressor to *gal* DNA. Our data do not determine the extent or the size of the operator. A more complete definition of the *gal* operator will be obtained by direct protection experiments against chemical modification of the DNA and by the isolation and sequencing of additional mutants. Such experiments are in progress. Interestingly, in three of the mutants two adjacent base pairs have been changed. The high frequency of this type of mutation is probably due to the method of mutagenesis—UV light.

The location of the *gal* operator contrasts with the location of the operators in other operons. The *lac* repressor protects about 20–22 base pairs, between –3 and +20 (ref. 6). The *trp* repressor interacts with a segment which includes at least the portion between –5 and –17 (ref. 16). At each of two λ promoters, λP_R and λP_L , the *lambda* repressor binds with decreasing affinity to three adjacent operators. The λ operators which are closest to the respective initiation sites have the highest affinity for the repressor⁸. In each of these systems the repressor thus binds at close proximity to the transcription initiation site and thereby prevents directly RNA polymerase from forming a stable pre-initiation complex. The location of the *gal* operator suggests a different mechanism for repression.

Examination of the CRP binding site in *lac* and a segment in *gal* located about the same distance from the cyclic AMP–CRP

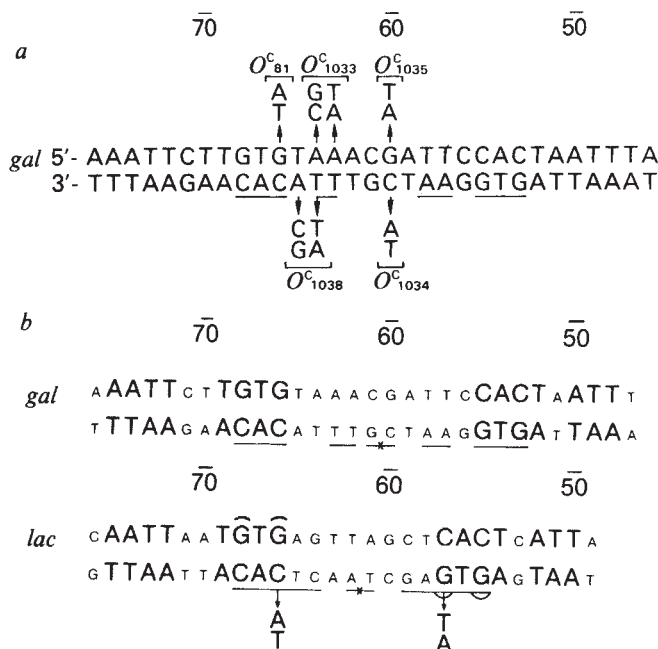


Fig. 4 a, Summary of the base changes caused by 5 different *galO^c* mutations. b, Comparison of the *lac* CRP site with a similarly located segment in *gal*.

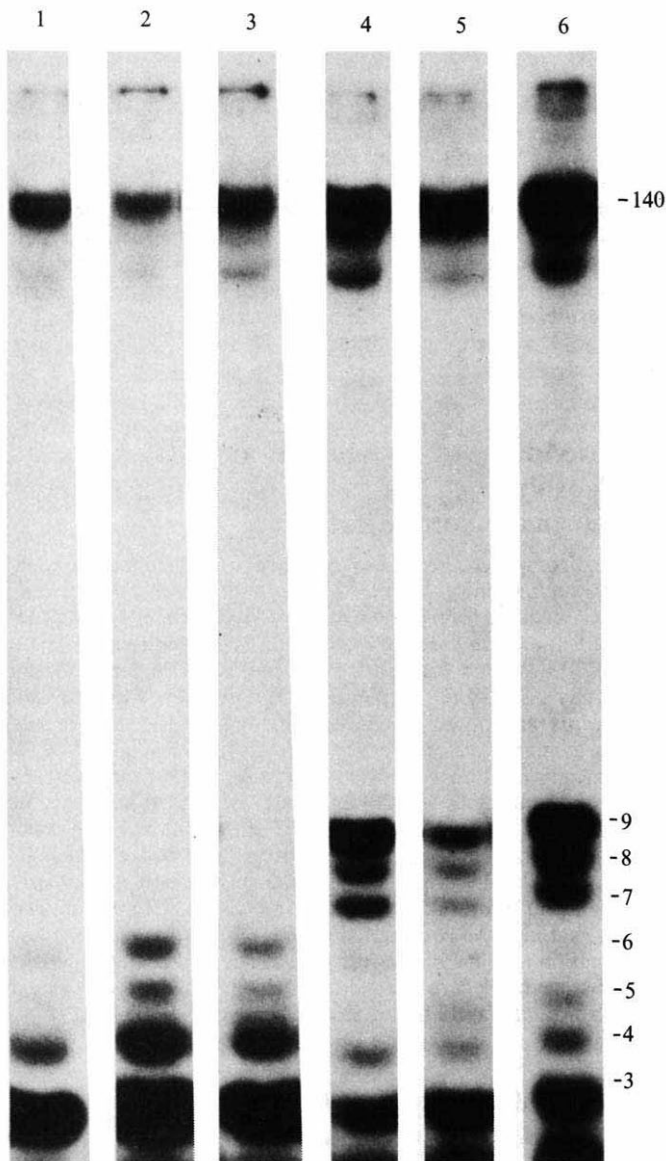


Fig. 5 Effect of the *gal* repressor on initiation of transcription at S1 and S2. The *gal* repressor was purified from an *E. coli* strain containing the plasmid pGR4, a derivative of plasmid pBR313¹⁸ in which the *galR* gene was introduced by *in vitro* recombination (R. di L., unpublished results). The repressor was purified according to a published procedure⁵, except that the affinity chromatography step was substituted with chromatography on single stranded DNA cellulose (R. di L., unpublished). Transcription reactions were carried out in a final volume of 50 μ l containing 1 μ mol Tris-HCl (pH 7.9), 5 μ mol KCl, 0.15 μ mol MgCl₂, 5 nmol EDTA, 5 nmol DTT, 2.5 μ g bovine serum albumin, 0.9 μ g CRP, 0.5 μ g RNA polymerase, and 20 ng of the 235 base pair *Hha*I fragment of Fig. 1. After 10 min of incubation at 37 °C, heparin 5 μ g was added to inactivate unbound RNA polymerase and transcription was started 1 min later by adding 5 μ l of a solution containing 1 mM ATP and 100 μ M each of GTP, CTP and [α -³²P]UTP (10–20 Ci mmol⁻¹). Where indicated, cyclic AMP (10 mmol), *gal* repressor (0.9 μ g) and D-fucose (1 μ mol) were added before the addition of heparin. After 10 min at 37 °C reactions were terminated by adding 0.25 ml of 0.1 M Tris-HCl pH 7.4, 0.2% SDS, 10 mg ml⁻¹ tRNA, and 10 mM EDTA. After extraction with phenol, the RNA was precipitated with ethanol, redissolved in 10 M urea, 0.025% bromophenol blue, 0.025% xylene cyanol FF and electrophoresed on an 18% polyacrylamide gel in 7 M urea¹¹. Lanes 1–3: no cyclic AMP added; lane 1, no repressor; lane 2, repressor alone; lane 3, repressor and D-fucose. Lanes 4–6: cyclic AMP added; lane 4, no repressor; lane 5, repressor alone; lane 6, repressor and D-fucose. Numbers on the right of the figure indicate the size of the RNAs in nucleotides.

startsite of transcription shows striking similarities both in sequence and symmetry (see Fig. 4b). In *lac*, sequence data of CRP site mutants and chemical protection experiments indicate that the symmetry in Fig. 4b is probably recognised by CRP. In *gal* all *O*^c mutants which we examined are clustered between -66 and -60, so lie within the same region. Hence one possible mechanism for *gal* repression could imply an interaction of the *gal* repressor with the same site which is recognised by CRP: the *gal* repressor could block *P*₁ transcription by simply preventing CRP binding to DNA. The following results, however, indicate that despite the similarities in sequence and symmetry CRP does not interact with this segment in *gal*. (1) All *O*^c mutations which we have examined exhibit the same degree of stimulation of *P*₁ activity by cyclic AMP–CRP and inhibition of *P*₂ function by these factors as wild-type DNA. More quantitative data were

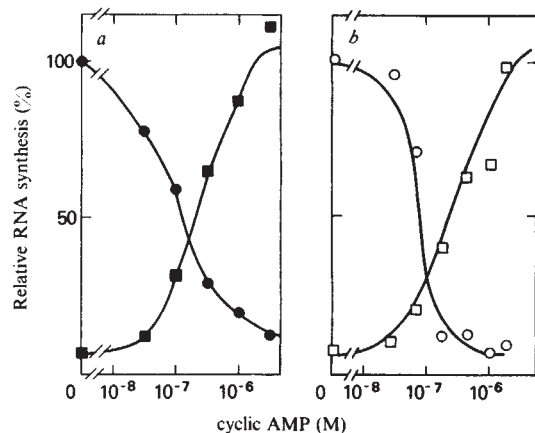


Fig. 6 Comparison of cyclic AMP effects on *P*₁ and *P*₂ transcription for wild-type *gal* DNA and mutant *O*₈₁*gal* DNA. Conditions for transcription and analysis of the transcripts on 18% polyacrylamide–7 M urea gels were as described in Fig. 5 legend. Radioactive bands, visualised by autoradiography specific either for *P*₁ or *P*₂ transcription, were excised from the gel and counted. A 9-nucleotide-long transcript is specific for *P*₁, whereas a 6-nucleotide-long transcript is specific for *P*₂. *a*, Wild-type *gal* DNA: ■, *P*₁ RNA; ●, *P*₂ RNA. *b*, *O*₈₁*gal* DNA: □, *P*₁ RNA; ○, *P*₂ RNA.

obtained with one *galO*^c mutant (*O*₈₁^c). This mutation lies at the same location within the 'common' symmetry and causes the same base change as a *lac* CRP binding site mutation¹³ (see Fig. 4). In the mutant *gal*, the cyclic AMP–CRP concentration dependency for *P*₁ activation (and for *P*₂ repression) is indistinguishable from that obtained with wild-type DNA. Thus a similar mutation which severely reduces CRP-dependent promoter activity in *lac* has no effect on this function in *gal*, indicating that unlike in *lac* this site is not essential for CRP function in *gal*. (2) Protection experiments indicate that CRP binds between -50 and -25 in *gal* DNA and not to the segment which is analogous to the *lac* CRP binding site (T.T. and B.deC., unpublished results). (3) Replacement of the DNA to the left of -60 with an unrelated DNA sequence does not change the concentration dependency for cyclic AMP–CRP stimulation of *P*₁ and repression of *P*₂ (T.T. and B.deC., unpublished results). Thus CRP is capable of activating gene transcription by interacting at a different location in different CRP-dependent promoters.

Our results clearly indicate that binding of the *gal* repressor to *gal* DNA does not prevent the inhibition of *P*₂ transcription by cyclic AMP–CRP. One possible explanation would postulate the existence of two binding sites for cyclic AMP–CRP, one for

P1 stimulation, the other for *P2* repression. *gal* repressor would interfere with the binding of cyclic AMP–CRP at one site, not at the other. An alternative hypothesis to explain both the stimulatory and inhibitory effects of cyclic AMP–CRP is that the

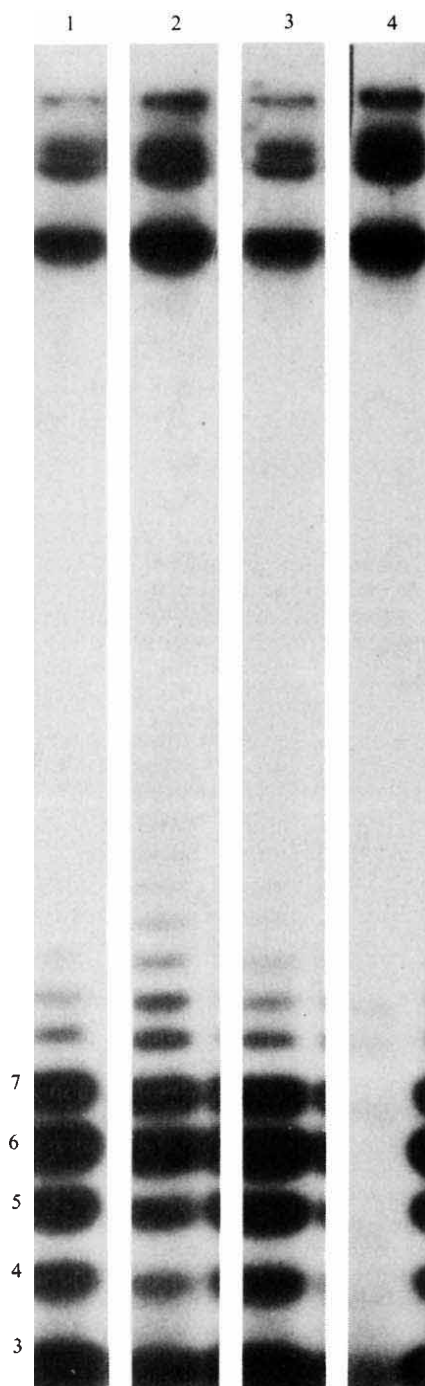


Fig. 7 Effect of nucleoside triphosphate (NTP) concentrations on transcription from S1 and S2. Transcription reactions and polyacrylamide gel analysis were as in Fig. 5 except for the NTP concentrations as described below. [α - 32 P]UTP (10–20 Ci mmol $^{-1}$) was used as radioactive precursor. Lanes 1 to 3: no cyclic AMP added. Lane 1: ATP 100 μ M, GTP, CTP and UTP each 10 μ M; lane 2: ATP, GTP, CTP and UTP each 100 μ M and 20% glycerol (v/v); lane 3: ATP, GTP, CTP and UTP each 100 μ M; lane 4: cyclic AMP added, ATP, GTP, CTP and UTP each 100 μ M.

interaction of cyclic AMP–CRP with a unique DNA binding site could create different protein–protein interactions which would facilitate the formation of a stable transcription initiation complex at *P1* and inhibit this reaction at *P2*. Our finding that the concentrations of both CRP and of cyclic AMP needed for half maximal stimulation of *P1* are identical to those which cause 50% repression of *P2* is consistent with the latter model. If we postulate such a unique and specific binding site for CRP, it is obvious that the *gal* repressor cannot simply prevent CRP from binding to this site, since CRP still inhibits *P2* transcription in the presence of *gal* repressor. We believe that *gal* repressor could block the productive interaction between CRP and RNA polymerase at *P1* without affecting the interaction between CRP and RNA polymerase at *P2*. We cannot exclude, however, that cyclic AMP–CRP interacts with RNA polymerase first and the cyclic AMP–CRP–RNA polymerase complex binds to *P1* requiring little or no specific interactions between cyclic AMP–CRP and DNA. The same cyclic AMP–CRP–RNA polymerase complex would be unable to bind to *P2*. The high specificity of binding of the cyclic AMP–CRP complex itself to *lac* DNA^{12,15} and to *gal* DNA (T.T. and B.deC., unpublished observations) does not favour such an hypothesis.

In vivo the *gal* operon can be induced by galactose or fucose even when cyclic AMP or functional CRP are eliminated from the cells by mutation. Both *galR* $^{-}$ mutants and cells harbouring many copies of a plasmid which contains an intact *gal* operator exhibit high levels of galactokinase synthesis whether cyclic AMP or CRP are present or not (see accompanying article⁹ and B.deC., unpublished observations). *galO* $^{+}$ mutants also show a high level of constitutive *gal* expression in the presence or absence of cyclic AMP–CRP. These *in vivo* results clearly indicate that the interaction between the *gal* repressor and the *gal* operator causes repression of both *P1* and *P2*. *In vitro*, however, the *gal* repressor blocks only the cyclic AMP–CRP dependent transcription from S1 but does not decrease initiation of transcription from S2. The repressor binds to *gal* DNA in the absence of cyclic AMP–CRP but does not prevent RNA polymerase from initiating transcription at S2. Hence, the *in vitro* results reproduce the *in vitro* regulation by the *gal* repressor when transcription starts from S1. In the absence of cyclic AMP or CRP, however, repression of *gal* transcription occurs *in vivo* but not, in our experimental conditions, *in vitro*. This could suggest that our defined *in vitro* system is lacking one or more elements responsible for the repression at S2 *in vivo*.

In earlier *in vitro* experiments using λ *gal* DNA as template it was found that cyclic AMP and CRP stimulated *gal* mRNA synthesis 5- to 10-fold¹⁶. *gal* mRNA was measured by DNA–RNA hybridisation and the majority of the RNA molecules which were analysed were of large size. We have shown here that initiation of *gal* transcription at S2 is in fact more efficient than at S1, but that even with elevated NTP concentrations RNA polymerase terminates transcription prematurely. Thus the low amounts of hybridisable *gal* mRNA found *in vitro* in the absence of cyclic AMP and CRP were probably due to a defect in elongation and not of initiation of transcription. Hence the high levels of *gal* expression which are observed *in vivo* in the absence of cyclic AMP or CRP could be due to a mechanism which would prevent premature termination of transcription. We propose that repression of *gal* transcription from S2, as is observed *in vivo*, could control the rate of premature termination.

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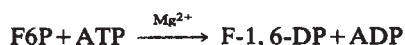
Structure and control of phosphofructokinase from *Bacillus stearothermophilus*

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The allosteric enzyme phosphofructokinase binds its substrate fructose-6-phosphate between two subunits of the tetramer, and allosteric effectors between another pair of subunits. The effector binding site accommodates both the activator and the inhibitor. The substrate cooperativity and allosteric control are mediated by these ligand bridges between subunits.

PHOSPHOFRUCTOKINASE (EC 2.7.1.11) catalyses the phosphorylation of fructose-6-phosphate (F6P) by ATP to form fructose-1,6-diphosphate in a reaction which controls the rate of glycolysis in the cell.



The enzyme has been isolated from a wide variety of sources (for reviews, see refs 1,2), and its activity is regulated by a variety of intracellular metabolites, depending on the source. The enzymes from muscle and from yeast have complex oligomeric structures with subunits of molecular weight 85,000 (refs 1,2) and 100,000 (ref. 3) respectively. In contrast, the enzymes from bacteria are much smaller, and have simpler control properties.

We have worked on the phosphofructokinase (PFK) from the thermophilic bacterium *Bacillus stearothermophilus*, which is a stable tetramer of identical subunits each of molecular weight 33,900. The complete amino acid sequence of 316 residues has been determined⁴. Preliminary kinetic analysis shows a sigmoidal dependence of activity on the concentration of the substrate F6P, allosteric activation by ADP, and inhibition by phosphoenolpyruvate (PEP) (H. Hengartner, unpublished). This allosteric behaviour is similar to that found for the enzyme from *Escherichia coli*, which has been explained with two states of the molecule, an active R-state and a less active T-state⁵. Both enzymes behave as K-systems, that is the two states differ in their affinity for the cooperative substrate F6P, but not in their catalytic rate k_{cat} . Unlike the eukaryotic PFKs, these bacterial

enzymes are not inhibited by ATP or citrate, nor activated by AMP. The relationship between the enzymes from prokaryotic and eukaryotic sources remains unclear. We have been looking for a stereochemical explanation of the enzyme activity and investigating the cooperativity of binding of F6P and the influence of the allosteric effectors on the activity. We present here a preliminary report on the structure and substrate binding to the enzyme, which suggests explanations for some of these phenomena.

X-ray structure determination

PFK was purified from cell extracts of *B. stearothermophilus* by affinity chromatography on AMP-Sepharose⁶ and ATP-agarose⁷. Large crystals suitable for X-ray diffraction were grown from ~2 M potassium phosphate pH 7.3 containing 2 mM fructose-6-phosphate, 1 mM dithiothreitol and 0.5 mM EDTA. X-ray photographs show that the crystals belong to space group I222 with cell dimensions 122.5 Å, 84.1 Å, 61.5 Å and with one subunit in the asymmetric unit. Native crystals were prepared for data collection by washing out the F6P with 2.5 M phosphate solution. Intensity data were collected to 2.4 Å resolution on a rotation camera for the native and three heavy atom derivatives, (*p*-hydroxymercuribenzoate, ethylmercury thiosalicylate, and Au(CN)₂⁻). An electron density map was calculated by the usual method of isomorphous replacement using phases calculated from the three heavy atom derivatives.

A phase improvement method suggested by Agarwal and Isaacs⁸ was tried, starting with the isomorphous replacement map. A model structure was created by distributing atoms of atomic number 7 through the high density regions of the map, such that the number of atoms in the asymmetric unit was approximately correct (~2,400) and they were not too close together. The positions and thermal parameters for these dummy atoms were refined by a diagonal least-squares procedure^{9,10}, and the calculated structure factors after eight cycles of refinement ($R = 0.255$) were used to calculate phases. The calculated phase probability distributions were combined with those from the isomorphous derivatives¹¹⁻¹³, and a second electron density map was calculated with these combined phases. Although the theoretical justification for this method is dubious, particularly at a resolution as low as 2.4 Å where the ratio of observations to parameters is only about 1.35, it does seem to sharpen the electron density in a useful way.

The two electron density maps were contoured on to small

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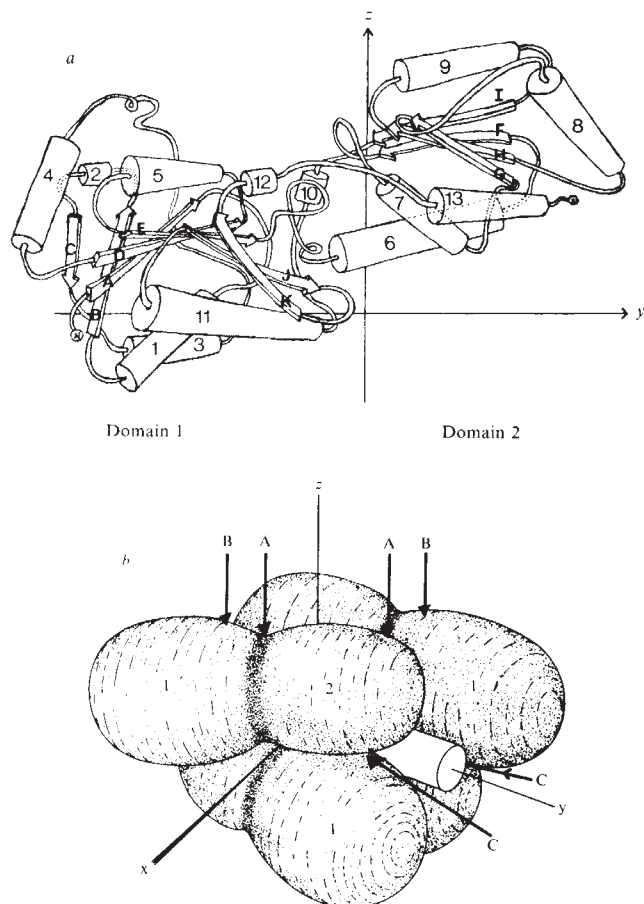


Fig. 1 *a*, Schematic drawing of the polypeptide chain of one subunit, viewed along the *x* axis. Arrows represent β -sheet strands (A-K) and cylinders represent α -helices (1-13). *b*, Schematic drawing of the tetramer of PFK. Each subunit is shown divided into two domains, numbered 1 and 2. The four subunits are related by three orthogonal dyad axes (222 symmetry), which coincide with the crystallographic symmetry axes. There is a solvent filled hole of ~ 7 Å diameter through the centre of the tetramer along the *y* axis, indicated by a cylinder. The positions of some of the sites A, B and C are marked, showing that sites A and C lie between subunits.

scale map stacks ($1 \text{ Å} = 2.5 \text{ mm}$). From these we could trace the course of the entire polypeptide chain, and locate nearly all the amino acid side chains. It was easier to trace the main chain through the map from the combined phases, but more recent model building into the density on a computer graphics system¹⁴ has shown that the shape of the electron density is better in the isomorphous map. Nevertheless, the procedure was useful in aiding the initial interpretation and improving some of the poorer regions of the density. Part of the isomorphous replacement map is shown in Fig. 2, showing the top of domain 1 and the central β -sheet of domain 2. The interpretation of nearly all of this map was clear, particularly in the helices and the β -sheet regions. The positions and shapes of the side chains agreed with the chemical sequence, aromatic residues and methionines being unmistakable. In the internal parts of the structure, most of the main chain carbonyl groups could be seen as bulges on the electron density. A few of the loops on the surface were less clear, particularly the loops between helix 1 and sheet strand B, and between helices 3 and 4. Details of the crystallography will be published elsewhere.

The structure of the protein

This description is based on the approximate α -carbon coordinates measured from small-scale map at 2.4 Å resolution. The more precise model building into the electron density confirms

all the essential features. Figure 1*a* illustrates one subunit, and Fig. 3 shows the α -carbon chain in stereo with the subunit interactions, the substrates and effectors. Figures 4 and 5 are also schematic views of two halves of the tetramer.

The PFK subunit consists of two domains each of which has a central β -sheet sandwiched between α -helices, a type of fold which has been seen in a number of proteins (α/β class of proteins of Levitt and Chothia¹⁵). However, the topology of the β -sheet strand connections in both domains of PFK is different from that in any other known protein structure. The first domain (Fig. 1*a*) has seven strands of β -sheet, the central five being parallel, and the outer two antiparallel to the others. The second domain has four parallel strands. The two sheets point towards a deep cleft which forms the active site (see below). Starting from the amino terminus, the chain first forms the main part of domain 1, including five strands of sheet A to E, and helices 1-5, then crosses to the second domain through the long helix 6. There follows most of domain 2, then the chain returns to domain 1 for the last two sheet strands J and K. Finally an extended chain returns to domain 2 for the final helix 13. Domain 1 consists approximately of residues 1-131 and 251-301, and domain 2 of residues 132-250 and 302-316. This insertion of one domain into the middle of the polypeptide chain of another is unusual in domain proteins: in most cases the domains are segregated along the chain, although the final crossing to the other domain for a C-terminal helix is more common (for example, glyceraldehyde-3-phosphate dehydrogenase^{16,17} and phosphoglycerate kinase¹⁸). Other proteins with such inserted domains include pyruvate kinase¹⁹ and the *E. coli* arabinose-binding protein²⁰. All but one of the connections between successive parallel β -strands are right-handed^{21,22}; the exception is the connection between strands E and J, where the connecting loop contains the whole of the second domain, about 157 residues.

Each subunit forms close contacts with only two of the other subunits in the tetramer. The interaction across the dyad along *z* is shown in Figs 3*a* and 4: the end of helix 6 is tightly packed against the neighbouring subunit, and the β -strands I interact across the dyad axis. The relative twist of these strands is in the wrong direction for extensive antiparallel β -sheet interactions, but two hydrogen bonds are formed across the dyad axis. The other subunit contact, across the dyad along *x*, is shown in Figs 3*b* and 5. The main interaction is the packing between the three helices 1, 3 and 11, and three helices 6, 7 and 13 in the other subunit. Also the bend between the β -strands J and K is in contact with its counterpart across the dyad. Figure 3*c* is a view of the tetramer from the end of the subunits, along *y*, and shows both of these subunit contacts. A solvent-filled hole $\sim 7 \text{ Å}$ in diameter passes through the middle of the tetramer separating the pairs of subunits which do not interact.

Table 1 Compounds binding to the three binding sites A, B and C

Active site		Effector site
A	B	C
F6P	ADP/Mg or Mn	P _i
P _i	AMPPNP/Mg or Mn	ADP/Mg or Mn (activator)
(FDP)	(ATP)	PEP (inhibitor)

Compounds in brackets have not been observed, but are assumed to bind in these sites. Binding of ligands other than P_i in site C is only observed in crystals which have been transferred to 1.25 M sodium citrate (pH 7.3), or to 2.5 M potassium tartrate (pH 7.7). In the citrate solutions, and tartrate solutions containing ADP, the phosphate ion in site C is displaced, but the phosphate in site A remains. Transfer of the crystals to tartrate solution in the absence of other ligands causes the crystals to crack and change their space group, suggesting that a structural change has occurred. No binding of tartrate or citrate ions is apparent.

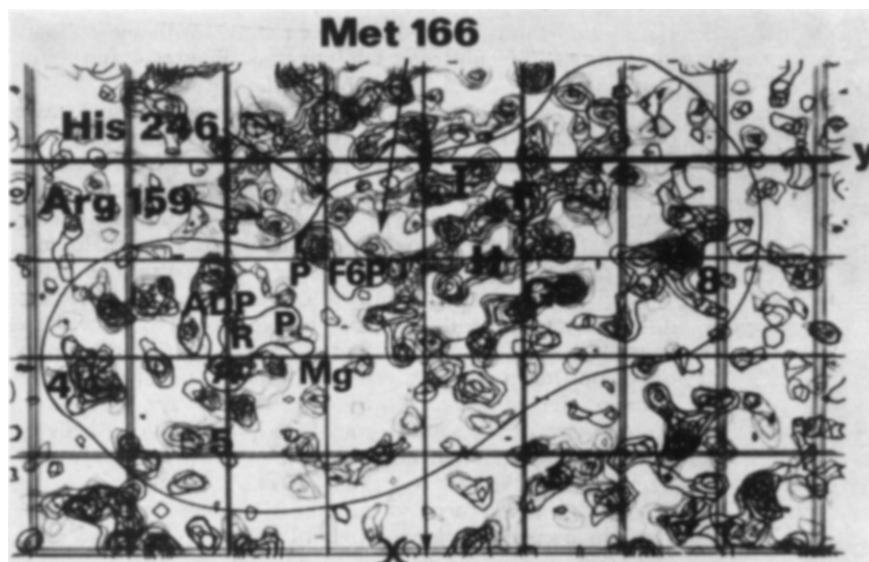


Fig. 2 Sections $z = 20/76$ to $26/76$ of the isomorphous replacement electron density map. This view corresponds to those in Figs 3a and 4. The grid lines are at intervals of 10 Å. One subunit is outlined: part of the subunit related by the dyad axis marked on the z axis is shown at the top. Outlines of the difference electron density for F6P and ADP are shown. The F6P outline encloses the high density peak representing the inorganic phosphate ion in the native map (labelled P). The ADP outline is labelled A for adenine, R for ribose, P for the α -phosphate, and Mg for the magnesium ion. Three of the side-chains which bind the F6P molecule are indicated: His 246 and Arg 159 (from the upper subunit) bind the phosphate together with Arg 240, which is above the sections shown. Met 166 is one of the residues forming the pocket for the fructose ring. The four β -sheet strands of domain 2 are labelled F, G, H, I, and three α -helices are labelled 4, 5 and 8 (compare Fig. 4).

Binding studies

The binding of several compounds has been studied in the crystal, mostly using data to 6 Å resolution, but with one set of data to 2.4 Å resolution. There are three binding sites per subunit, sites A and B forming the active site and site C which appears to be the effector site. The molecules which bind to these sites are summarised in Table 1. Site A binds F6P in the crystals as grown, but this is replaced by PO_4^{3-} when F6P is washed out to make the native crystals. The phosphate ion is the highest peak in our electron density map (Fig. 2). Presumably this site should also bind fructose-1,6-diphosphate (FDP), but crystals soaked in this compound show only weak binding. Site B binds ADP with either Mg^{2+} or Mn^{2+} , but difference maps from crystals soaked in ATP show density identical to ADP, perhaps because of hydrolysis of ATP by the enzyme. The ATP analogue AMPPNP (5'-adenylylimidodiphosphate) binds in this site also with either Mg^{2+} or Mn^{2+} , and shows additional electron density which presumably represents the γ -phosphate group. Site C appears to be the effector site, and binds a phosphate ion in our native crystals: this ion also shows clearly in the electron density map. This site binds both the activator ADP and the inhibitor PEP.

Details of the binding sites

Figures 4 and 5 show the binding of the substrate pair F6P and ATP/Mg in the active site, and the activator ADP/Mg in the effector site. These are superimposed on diagrams of two subunits from the tetramer, together with those side chains which are in contact with the ligands. The positions of the bound molecules were taken from a difference map at 2.4 Å resolution from crystals soaked in F6P, ADP and Mg in 2.5 M potassium tartrate. This map showed clear density for F6P in site A and ADP/Mg in the effector site C, but the ADP/Mg in site B was less clear and only about half occupied: there was no electron density for the β -phosphate. Models of the three ligands were fitted to the electron density on a small Richards' box. The drawings show ATP in the active site, based on the 2.4 Å resolution ADP density and the γ -phosphate position from a difference map between AMPPNP and ADP at 6 Å resolution. The magnesium ions are clear in the difference electron density and correspond to their positions deduced from the 6 Å resolution difference map between Mn^{2+} and Mg^{2+} .

Site A. The notable feature of this site is that the 6-phosphate group of F6P (and the phosphate ion in the native crystals) is

bound by amino acid side chains from two adjacent subunits, His 246 from one subunit, and Arg 159 and Arg 240 from the other subunit (Fig. 4). Thus the cooperative ligand F6P bridges between two subunits. The 1-hydroxyl is close to the γ -phosphate of ATP, and appears to be hydrogen bonded to Asp 124. This residue may act as a base catalyst for the reaction by increasing the nucleophilicity of the 1-OH group. This phosphorylation site lies in the boundary between the two domains of the subunit, while the groups which bind the sugar ring of F6P (Arg 249, Met 166, Glu 219 and Asp 124) come from domain 2 of the subunit (the right-hand domain in the lower subunit in Fig. 4).

Site B. The conformation of ADP (and hence ATP) in this site is not very clear from our current maps, nor are all the interactions with side chains. The magnesium ion binds in the same position with both ADP and AMPPNP (and therefore presumably with ATP). In ADP it binds to the α -phosphate and possibly to the invisible β -phosphate: in ATP it probably bridges the α - and β -phosphate groups. The ATP molecule is bound to domain 1 with the γ -phosphate near the domain boundary: the only residue involved from domain 2 is Arg 168, which becomes ordered near the phosphates when the nucleotide is bound. Asp 100 may be involved in the binding of Mg^{2+} .

Site C. While the substrate F6P is bound between the subunits related by the z dyad axis, the effector is bound in a cleft between subunits related by the x dyad. The key interaction with ADP seems to be with the β -phosphate group, which replaces the phosphate ion bound to this site in the native crystals. This phosphate is bound by three arginine residues, Arg 151 from the upper subunit in Fig. 5, and Arg 21 and Arg 25 from the lower subunit. The ribose is bound by His 212 and Thr 155. The Mg^{2+} ion bridges the α - and β -phosphates of ADP, but its only interaction with the protein seems to be with a main chain carbonyl group. The diphosphate of ADP points into a cleft, which is consistent with the specificity of the site for ADP rather than AMP or ATP, and its lack of specificity for the base⁵. The phosphate group of PEP appears to bind in the same place as the β -phosphate of ADP. This site is best considered as a phosphate binding site, rather than a nucleotide binding site.

Discussion

This structure of *B. stearothermophilus* PFK seems to correspond to the active R-state of the Monod, Wyman, Changeux model^{5,23}. A structural change caused by transferring the crystals to tartrate solution suggests that a second conformational state exists. All the structures we have been able to look at contain either phosphate ion or F6P bound in site A, and we

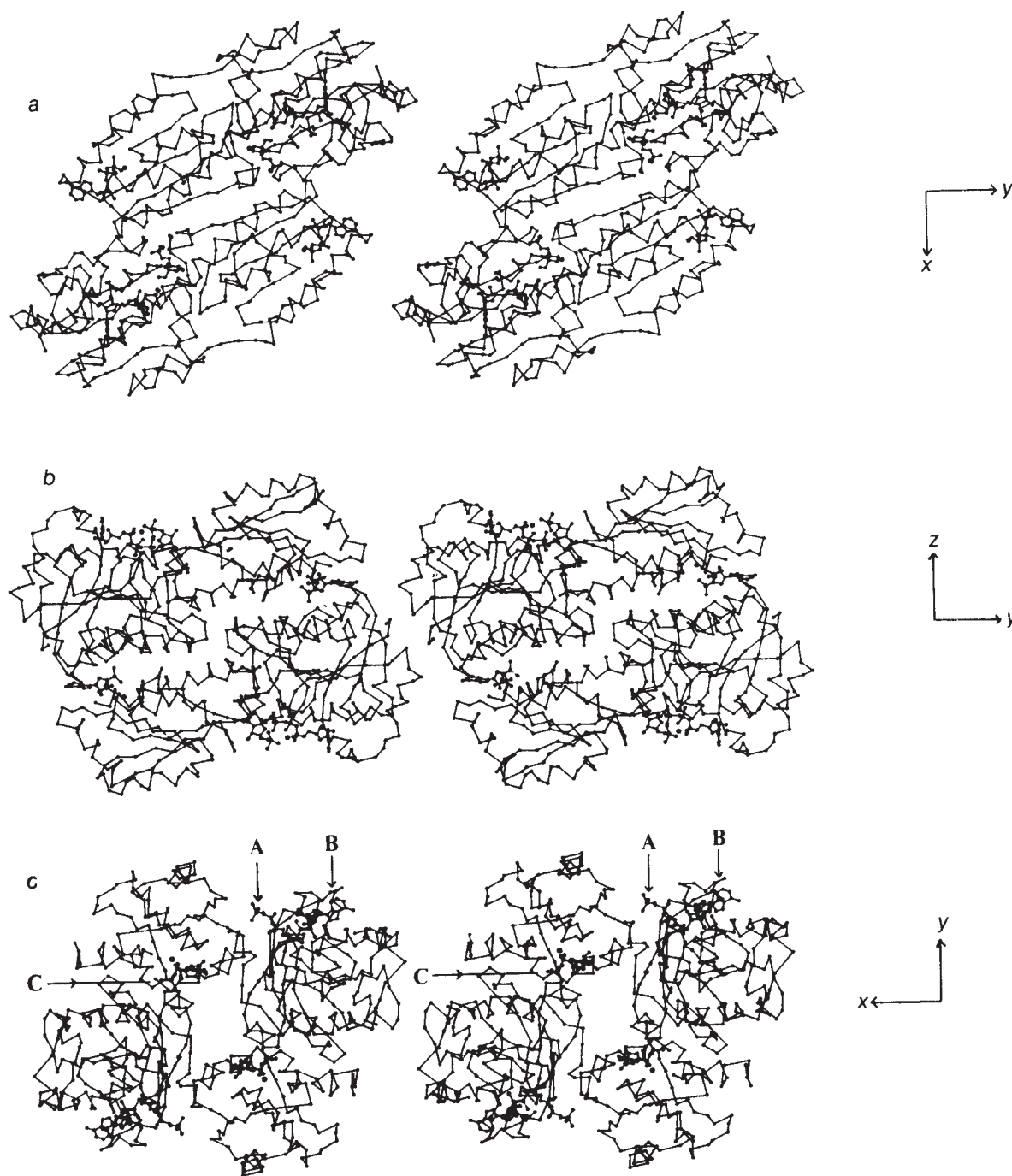
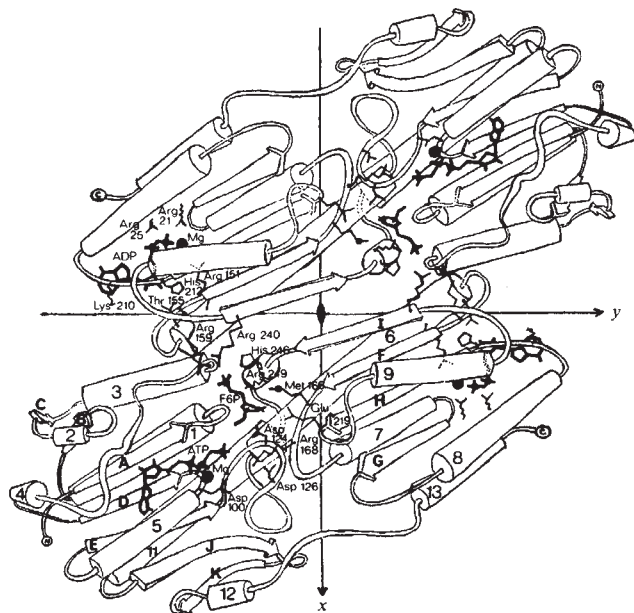


Fig. 3 Orthogonal stereo views of the α -carbon atoms of PFK together with the substrates F6P and ATP/Mg, and the activator ADP/Mg, viewed towards the centre of the tetramer. *a*, The upper two subunits of the tetramer viewed along the *z* axis (compare Fig. 4). *b*, The upper two subunits viewed along the *x* axis (compare Fig. 5). *c*, The upper half of the tetramer viewed along the *y* axis. Only half of each subunit is shown. The three ligand binding sites are indicated.

suggest that the removal of these ligands triggers the structural change to the less active T-state. Without knowing its structure, we cannot account in detail for the difference in affinity for ligands between the two states, but the nature of the binding site suggests a plausible explanation. The F6P site bridges two subunits, so a change of the relative position of these subunits could remove the two arginines 159 and 240 from the F6P site, reducing its affinity for F6P without changing the mode of binding. As the active site of PFK lies between the two domains of the structure, it would seem likely that any relative movement of these domains would change the catalytic rate, k_{cat} , by moving

the catalytic groups. As a change of k_{cat} is not observed, a more plausible model for the allosteric transition is a rearrangement of essentially rigid subunits into a different quaternary structure. This would contrast with other enzymes in which two domains within a subunit move on binding of substrates: these include hexokinase²⁴, glyceraldehyde-3-phosphate dehydrogenase (A. J. Wonacott, personal communication) and probably phosphoglycerate kinase¹⁸.

The effector site lies at the interface between another pair of subunits; as it binds both activator and inhibitor, the affinity for these molecules must change in opposite senses during the



allosteric transition. The local changes around this site are different with binding of ADP and of PEP, but we cannot explain the relative affinities of the two conformations without knowing the structure of the T-state.

Each subunit in the tetramer has an interface with two other subunits, one of these is bridged by F6P, the other by the effector (see Fig. 3c): thus catalysis and control require the whole tetramer. Figure 4 shows that the active site is close to the effector site, and the part of the enzyme that lies between the sites, particularly residues 151 to 159, has side chains pointing in one direction to the F6P site and in the opposite direction to the effector site. Thus binding of a ligand in either site could affect

All three binding sites in PFK bind phosphate groups, but there are interesting differences between them. Sites A and C both bind an inorganic phosphate ion as well as their specific ligands, and they have a clear electrostatic mode of binding: site C binds the phosphate with three arginine residues, and Site A with two arginines and a histidine. In contrast, site B does not bind inorganic phosphate, and does not have such a clear positively charged nature, although arginine 168 is close and the Mg^{2+} ion is bound to the α - and β -phosphates of ADP and ATP. The phosphates in this site are at the amino end of helix 5, and it has been suggested that the dipole of an α -helix provides approximately half a positive charge at its amino end²⁵. The difference between the three sites may be related to the requirements for binding at sites A and C, compared to binding and catalysis at site B. Site C is unusual in that it lies at the amino end of a β -sheet. In most proteins with parallel β -sheets, the binding sites for all types of ligand are at the carboxy end of the sheet (although the secondary ADP site in pyruvate kinase is at the amino end of the β -sheet barrel¹⁹). However, this effector site C is required by its function to be in the subunit interface, and this requirement presumably outweighs other preferences.

Over the past few years, there has been much discussion about the similarities between the tertiary structures of some proteins and whether these relationships are due to the rules of polypeptide folding or to evolutionary homology²⁶⁻²⁹. Among proteins with central β -sheets (α/β proteins), four different dehydrogenases resemble each other in one of their two domains. In contrast, five different kinases (adenylate kinase³⁰, hexokinase³¹, phosphoglycerate kinase^{18,32-34}, pyruvate kinase¹⁹ and PFK) show much less similarity. Although they all contain domains built around parallel β -sheet, the topological arrangement of these sheets are different, and their mode of binding substrates is different. These differences suggest that these kinases do not share a common ancestor.

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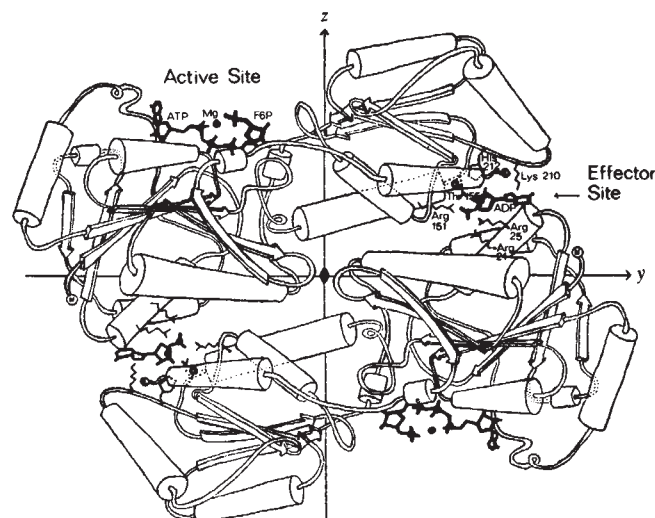


Fig. 5 Schematic view of two subunits along the *x* axis towards the centre of the tetramer. The substrates and activator are shown, and the relevant side chains around the effector site.

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letters

SS433—a massive black hole?

THE peculiar emission line object SS433 has recently been the subject of interest following new spectrophotometric observations by Margon *et al.*¹, and previous suggestions identifying it with the supernova remnant (SNR) W50 (ref. 2) and also with the X-ray source A1909+04 (ref. 3). Margon *et al.*¹ report "three very strong (equivalent widths 50–150 Å) broad emission features in the green, red and IR, which change in intensity, profile and wavelength daily." In this note we propose that the reported red and IR lines (at ~6,000 Å and ~7,400 Å, respectively) are Doppler-shifted H α lines which are emitted from a ring of matter orbiting around a ~10⁶ M $_{\odot}$ black hole. (The green line at ~5,200 Å may be the redshifted H β line.) The ring itself may be part of an accretion disk and its radius is ~20–25 gravitational radii ($R_g = 2GM/c^2$). The intensity of an emission line per observed Doppler-shifted frequency interval in such a ring is maximal at the two extreme ends of the spectrum⁴, in any case of emission constant along the ring. These maxima are quite sharp; in the non-relativistic case, we have

$$dI_{\nu}/d\nu \propto \{\nu_0^2 - (\partial\nu)^2\}^{-\frac{1}{2}}$$

where $\partial\nu$ is the frequency shift and ν_0 depends on the radius of the ring and on its inclination with respect to us. Also, due to the gravitational redshift in our case, the centre-of-mass of the red and IR lines emitted from such ring is additionally redshifted.

It is difficult to determine the relative intensity of the Doppler-pair members observationally, because of the uncertainty in the reddening out to SS433; if one takes $A_v \sim 7$ mag, as is reasonable for a distance of ~3.3 kpc in the galactic plane, the data of Margon *et al.* are consistent with both members having roughly equal intensities or even having the blueshifted member slightly stronger, as expected for such a model⁴. Also, to match these observed shifts¹, the plane of the ring should be inclined towards us⁴. Our numerical calculations for a model line emitted from such a ring are consistent with the structure of the observed pair in Fig. 3 of ref. 1 for inclination angles of 45–60°.

Accumulated results of observations (J. Katz, personal communication) of the above and other shifted lines from SS433 show the following characteristics:

(1) Let $B = (\lambda_{\text{blueshifted}} - \lambda_0)/\lambda_0$, $R = (\lambda_{\text{redshifted}} - \lambda_0)/\lambda_0$ and let c be the velocity of light, then, $\frac{1}{2}c(R-B)$ has now varied between ~10,000 km s⁻¹ and ~40,000 km s⁻¹. These high velocities strongly point to processes in a region of high gravitational field, near a neutron star or black hole.

(2) During these variations of B and R , the centre-of-mass (COM) of the lines ($\frac{1}{2}c(R+B)$) remains fairly constant⁵, redshifted at ~10,000 km s⁻¹. This poses severe difficulties on models in which the changes in R and B are due to velocity magnitude changes alone (rather than to changes in angle, such as a precession of the ring⁶, say, which is suggested by a possible periodicity in the data^{5,7}). It also indicates that the emission region for the shifted lines remains at a fairly constant gravitational redshift (that is, distance from the compact object).

(3) The shifted lines are accompanied by an unshifted line. This poses a difficulty on any interpretation of the shifted COM as an overall motion of SS433.

(4) The shifted lines are quite narrow, $(\delta\lambda/\lambda)/B_{\text{max}} \leq 10\%$, suggesting a reasonably narrow emission region.

(5) Let the line-emitting region be of surface area $4\pi R^2$. Then its brightness temperature T_b must satisfy $T_b > 10^9$ K $(R/10^9 \text{ cm})^{-2}$. This poses severe difficulties on neutron star models and on models having black holes with $R_g \leq 10^{11}$ cm ($M \leq 3 \times 10^5 M_{\odot}$), particularly with no observational evidence for coherent emission.

(6) In several of the H α Doppler-shifted pairs¹, one line seems to be the mirror image of the other (imaging on the λ axis, mirror located at the COM). This indicates a correlation between the geometries of the two emission regions, such as that found in emission from a ring. In pairs observed at other times, no such symmetry was found (J. S. Gallagher, personal communication), suggesting that the emission region has variable structure, or that its appearance to us is variable.

We infer from these considerations that the compact object, around which the emission takes place, cannot be a neutron star or a light black hole, but rather the above mentioned ~10⁶ M $_{\odot}$ black hole. An optically thick accretion disk around such a black hole has indeed most of its flux coming from such ring, at UV and visible frequencies⁸. Note, that if the centre of the SNR W50 is at distance ≥ 10 pc from SS433 (ref. 1), the disk may be fed by the SNR (although interstellar matter, if its density near the black hole is close to its average value in the Galaxy, can do the same); for typical values⁹ of the particle density (~1 cm⁻³) and velocity (~100 km s⁻¹) in such SNR, the feeding is at a rate of $\leq 10^{-7} M_{\odot}$ per yr, giving a total luminosity of $\leq 10^{39}$ erg s⁻¹ if the disk is in steady state. This is consistent with the observed optical luminosity, which seems to indicate that $M_{\odot} \leq -3.5$ (ref. 1).

The unshifted H α line¹ could arise from reprocessing of some of the $\leq 10^{39}$ erg s⁻¹ optical and UV radiation from the black hole, in the rim of the disk^{10,11}. This suggests that SS433 might be a strong UV source and that the shifted lines may also be due to reprocessed UV radiation.

Our model also suggests that the whole system may be part of an old globular cluster.

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Hard X-ray spectrum of Cyg X-1

THE three most likely black hole candidates known in X-ray astronomy are Cyg X-1, Circ X-1 and V 861 Scorpii¹. Cyg X-1 has a key role as it is the best studied of these objects. Unfortunately, the evidence for its being a black hole is entirely circumstantial; namely, the mass of the compact object and its observed short time variability. The spectrum of Cyg X-1 is also very different from those of accreting neutron stars, but it is similar to spectra of extragalactic sources which may contain massive black holes. Eardley *et al.*² have recently reviewed the existing evidence. Here we consider only the X-ray spectrum and the source luminosity. This work was motivated by the spectral measurements with very high statistical accuracy of Cyg X-1 made in the hard X-ray range by the AIT/MPI group³. Progress has been made in Moscow in calculating source spectra expected from hot accretion disks. Thus, there is a new basis for a comparison between observational results and theoretical predictions.

Measurements by numerous rocket, satellite and balloon experiments have shown that the Cyg X-1 spectrum is variable on different time scales (see ref. 4). In particular, the source exhibits 'flares' and transitions between high and low spectral states^{5,6}. Matteson *et al.*⁷ stressed that despite this variability most of the observed spectra lie near a 'normal' spectrum which can be characterised by a power law.

$$\frac{dN}{dE} = 3 \times 10^{-2} \left(\frac{E}{10 \text{ keV}} \right)^{-1.6} \quad (\text{photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}) \quad (1)$$

for $2 \text{ keV} \leq E \leq E_b$, while beyond E_b the spectrum steepens significantly. A range of values has been quoted for this break energy: $E_b = 32 \text{ keV}$ (ref. 8), 85 keV (ref. 7), 115 keV (ref. 9) and $\geq 150 \text{ keV}$ (ref. 10). During the past 10 yr the source has apparently spent most of its time ($\sim 90\%$) in this normal spectral state which has also been called the 'low' or 'non-flaring' state. The 'high' state in which the source is seen temporarily⁵ is characterised by a steep spectrum in the 2–10 keV range. As far as the break energy of the normal spectrum is concerned, it is not clear to what extent the quoted differences are due to the difficulty in positioning a 'spectral break' in the presence of statistical and systematic errors. On the other hand, the break energy is a very important parameter, as in the current comptonisation models it is related to the electron temperature of the scattering electrons (see ref. 2).

The hard X-ray spectrum of Cyg X-1 has been measured with high accuracy by the balloon group of the Astronomisches Institut Tübingen and MPI Garching on three different flights: 20 February 1975; 2 May 1976 and 20 September 1977. These measurements cover the spectral range from 15 to 150 keV. Figure 1 shows the data obtained on 20 September 1977 which have unprecedented accuracy.

The spectra taken during these three flights agree with each other to within $\sim 30\%$ and lie very close to the normal spectrum quoted above. They all show a steepening below 100 keV (ref. 3). Furthermore, they agree both in spectral slope and absolute intensities (within $\sim 40\%$) with high quality rocket spectra taken by the GSFC group on 4 October 1975¹¹. All these data suggest that the normal state has a more definite intensity (to $\leq 40\%$) than previously assumed.

The X-ray emission of Cyg X-1 has been explained in terms of disk accretion on to a black hole^{12–14} which is determined by the following parameters¹³: (1) the mass of the black hole $m = M_x/M_\odot$; (2) the accretion rate $\dot{m} = \dot{M}/\dot{M}_{\text{edd}}$ where $\dot{M}_{\text{edd}}$ is the Eddington critical accretion rate; (3) the turbulence parameter α .

At small accretion rates ($\dot{m} \ll 1$) the disk is stable but cool and unable to produce the observed hard X-ray spectrum. If the

accretion rate (onto a Schwarzschild black hole) becomes

$$\dot{m} \geq \frac{1}{50} (\alpha m)^{-1/8} \quad (2)$$

the radiation pressure will exceed the gas pressure in the inner part of the disk¹³ which leads to both secular¹⁵ and thermal^{16,17} instabilities. The unstable inner regions consist of hot and cold zones in pressure equilibrium. This means that the cold zones are dense and the production of low energy photons by bremsstrahlung and other collisional mechanisms is very effective. At the same time, these mechanisms are rather ineffective in the hot zones and hard X rays are mainly produced by comptonisation of the soft photons in the hot zone of the disk. This comptonisation is also the main cooling mechanism for the hot plasma.

As far as the physical nature and the detailed configuration of the hot gas component is concerned, various models have been discussed: a hot inner disk surrounded by a cooler disk^{18,19}, instability driven random outbursts of hot gas¹⁶, a hot corona surrounding a cool disk^{20–22}. The comptonisation of low energy photons in the high temperature electron clouds leads to the formation of power law X-ray spectra

$$J(\nu) \sim \nu^{-n} \quad \text{for } h\nu < kT_e \quad (3)$$

while above kT_e the spectrum cuts off roughly exponentially.

This was first shown in numerical calculations by Katz²³ for a non-relativistic plasma and by Pozdnyakov *et al.*²⁴ for relativistic and semi-relativistic plasmas using Monte Carlo methods. Shapiro *et al.*²⁵ found an approximate analytical formula for the spectral index of the power law spectrum.

Recently, Sunyaev and Titarchuk²⁶ derived an analytical solution for the comptonisation spectrum from a spherical, isothermal cloud in which a source of soft photons is embedded. This solution is valid for any spectral shape if the injected soft photons have an energy $h\nu_{\text{soft}} \ll kT_e$. The resulting high energy spectrum only weakly depends on the geometry of the clouds. For sufficiently high energies ($h\nu \gg kT_{\text{soft}}$), the analytical solution for the comptonisation spectrum can be written using the integral representation of a Whittaker function

$$J(E) = Ax^3 e^{-x} \int_0^\infty t^{n-1} e^{t \left(1 + \frac{t}{x} \right)^{n+3}} dt \quad (4)$$

where A is a constant and

$$\left. \begin{aligned} x &= \frac{E}{kT_e} = \frac{h\nu}{kT_e} \\ n &= -\frac{3}{2} + \left(\frac{9}{4} + \gamma \right)^{1/2} \\ \gamma &= \frac{\pi^2}{3} \frac{m_e c^2}{kT_e (\tau_0 + \frac{2}{3})^2} \end{aligned} \right\} \quad (5)$$

τ_0 is the Thomson optical thickness of the electron cloud. In the low frequency limit $x \ll 1$, one easily obtains from the integral representation of the Whittaker function that the spectrum is a power law (the same as obtained by Shapiro *et al.*²⁵):

$$J(E) \sim x^{-n} \quad (6)$$

Likewise in the high frequency limit $x \gg 1$ the spectrum is of the Wien-type

$$J(E) \sim x^3 e^{-x} \quad (7)$$

A fit of the spectrum given by equation (4) to the observed low state spectrum (Fig. 1) yields the following set of parameters:

$$kT_e = 27 \text{ keV} \quad (8)$$

$$\tau_0 = 5$$

Figure 1 shows the theoretical curve which is an excellent fit to

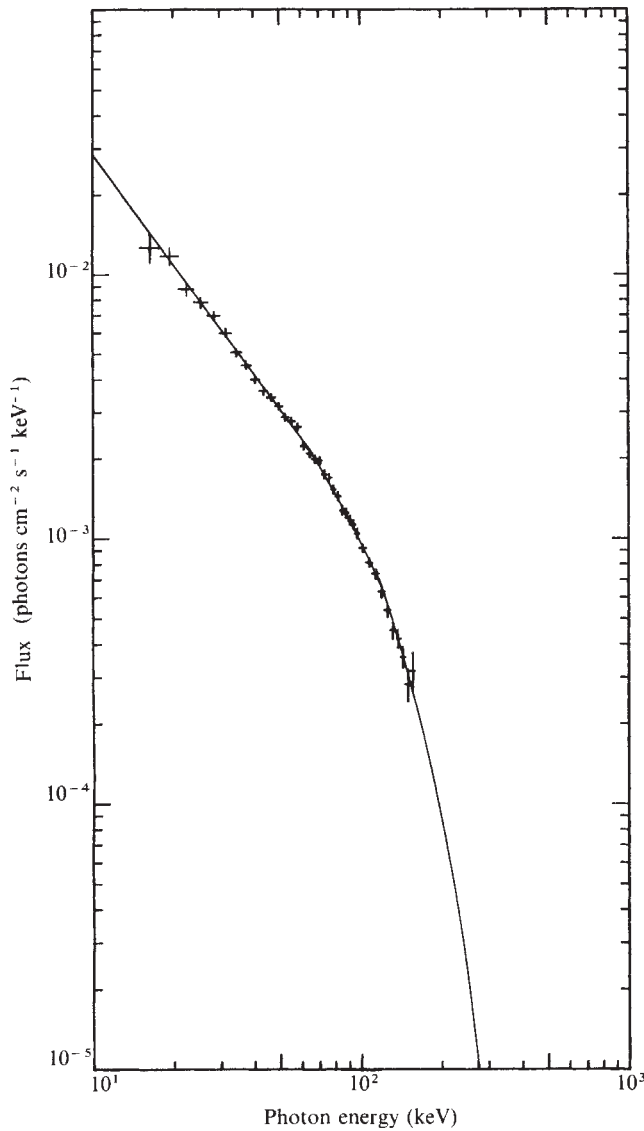


Fig. 1 Low state spectrum of Cyg X-1, observed on 20 September 1977 by the MPI/AIT balloon experiment³. The solid curve represents a photon spectrum expected by comptonisation of soft photons in an electron cloud with a temperature of 2.5×10^8 K and a Thomson optical thickness $\tau_0 = 5$.

the experimental data. Note that the temperature ($T_e \sim 3 \times 10^8$ K) is much less than that obtained in previous papers^{2,25} ($T \sim 10^9$ K).

We conclude that the comptonisation of soft photons by a hot gas gives a very good description of the observed low state spectrum. As the hard X-ray band (30–100 keV) contains the main part of the overall luminosity, this radiation must come from the inner region of the disk where the main energy release occurs. This region is quite compact (see ref. 13) and the larger and cooler outer regions do not contribute appreciably to the low state flux.

An integration of the spectrum for $E \geq 1$ keV yields

$$L_x = 2.3 \times 10^{37} \left(\frac{d}{2.5 \text{ kpc}} \right)^2 \text{ erg s}^{-1} \quad (9)$$

Thus, for a distance of 2.5 kpc (ref. 27) the observed luminosity is roughly 65 times below the Eddington critical luminosity for a $10 M_\odot$ black hole which is given by

$$L_{\text{edd}} = 1.3 \times 10^{39} \left(\frac{M_x}{10 M_\odot} \right) \text{ erg s}^{-1} \quad (10)$$

Therefore, the accretion rate $\dot{m}$ derived from observations is a factor ~ 1.3 lower than that required to obtain an unstable disk according to equation (2).

Before discussing this result we shall summarise briefly the requirements on the soft X-ray source feeding the comptonisation process. According to Sunyaev and Titarchuk²⁶

$$L_x = L_{\text{soft}} \left(1 + 2.9 \frac{kT_e}{\langle h\nu_{\text{soft}} \rangle} \right)^{1-n} \quad (11)$$

which for $L_x = 2.3 \times 10^{37} \text{ erg s}^{-1}$, $kT_e = 27 \text{ keV}$ and $n = 1.56$ yields

$$L_{\text{soft}} \approx 10^{36} \left(\frac{100 \text{ eV}}{\langle h\nu_{\text{soft}} \rangle} \right)^{0.44} \text{ erg s}^{-1} \quad (12)$$

What is the black-body temperature of the region where the main energy release occurs? For a Schwarzschild black hole this region has a radius of ~ 7 gravitational radii ($r \sim 2 \times 10^7 \text{ cm}$). The total luminosity of this region is given by $2\pi r^2 \sigma T_{\text{soft}}^4$. Equating this to L_{soft} as given by equation (12) and using

$$kT_{\text{soft}} = \frac{\langle h\nu_{\text{soft}} \rangle}{2.7}$$

we find

$$kT_{\text{soft}} \approx 100 \text{ eV} \quad (13)$$

The soft X-ray source must possess a luminosity of a few times $10^{35} \text{ erg s}^{-1}$ and a temperature of the order of 10^6 K .

We now reconsider why the observed X-ray luminosity is not sufficient (by a factor 1.3) to make the disk unstable. Within this framework of the black hole disk accretion models there are two solutions: (1) the discrepancy of a factor of ~ 1.3 is due to uncertainties in the observational and theoretical figures; and (2) there could be a large contribution to the radiation pressure due to unobservable (soft X and XUV) photons.

In the first case, the accretion disk must be quite close to the lower boundary (in L_x) of the region of instability. This is true if Cyg X-1 is a $10 M_\odot$ black hole, but also if it is a Kerr black hole for which the requirements on the mass accretion rate and total luminosity would be a little lower than given by condition (2). The marginal instability may lead to the total disappearance of the hard X-ray flux, if the mass accretion rate drops below the critical value. Although this critical mass accretion rate is not known precisely (it may be below the 'normal' rate by a factor of 2 to 3) it should represent a sharp threshold which is different for Schwarzschild and Kerr black holes. To our knowledge, there is no observational evidence for such 'off-states'. However, they might be very short (several seconds) if they are due to stochastic fluctuations of the accretion rate in the central parts of the disk. The detection of such short 'off-states' in the X-ray flux from Cyg X-1 and the other black hole candidates would be very important. In the long run, a precise determination of the threshold X-ray luminosity might yield information about the metric of the accreting black hole.

The other possibility is that the actual accretion rate and luminosity are considerably higher than the observed values, so that the disk is permanently unstable. This contribution to the overall luminosity must be substantial ($\geq 2 \times 10^{37} \text{ erg s}^{-1}$) and should consist of soft X-ray and XUV photons. The corresponding sources may be the cool parts of the disk, however, the required luminosity is much larger than that given by equation (12). Therefore, this case requires that the bulk of the soft flux freely escapes from the disk while only a small part (a few per cent) is subjected to the comptonisation process. A condition for this is, of course, that the hot gas clouds cover only part of the disk. Such large 'soft' luminosity of the disk would require a higher temperature ($kT_{\text{soft}} \sim 250 \text{ eV}$) than given by equation (13). Soft X-ray observations with high sensitivity may indicate a soft component in the low state spectra. Optical observations of the accretion disk would be important in this context as they may give information about the total energy flux which heats the peripheral parts of the disk²⁸.

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Search for short time-scale periodicity in the X-ray flux of 4U1700-37

THE first suggestion^{1,2} of the existence of a ~ 24 min periodicity in the X-ray light curve of 4U1700-37 was followed by the report³ of the detection of a ~ 97 -min period in the X-ray data from the SAS C satellite when pointing at the same source. We report here how a new recent Copernicus observation of 4U1700-37 was used to test the existence of a short-term modulation of the X-ray flux.

In July 1978 the Copernicus satellite observed 4U1700-37 for a complete binary cycle between day no. 191.2 and day no. 194.8 (from phase 0.48 of a cycle to phase 0.52 of the following, according to the ephemeris given in ref. 2) and again between day no. 195.7 and day no. 196.5 (phase 0.80-0.01). The data collecting time of the X-ray instrumentation^{4,5} was 62.9 s every 86.5 s (23.6 s dead time). The X-ray data are shown in Fig. 1, integrated in bins of ~ 1 h duration, after subtraction of the diffuse sky and particle background. A low amount of contamination (~ 20 counts per 62.9 s on average) from the nearby X-ray source Sco X-2 is present and visible during the 4U1700-37 eclipses. This would not affect the detection of a periodic modulation, if any, in the X-ray flux of 4U1700-37, but it does increase the noise in the search for periodicities.

For our analysis we used all the July 1978 Copernicus X-ray data relative to the non-eclipsed part of 4U1700-37 light curve. The data sampling interval of 86.5 s allowed the search

for periodicities down to ~ 3 min (corresponding to the Nyquist frequency). Because of the Earth occultation and the regions of high particle background where the detector was switched off the data sets are not regularly spaced, so we used the technique of Gray and Desikachary⁶ to Fourier analyse the data. We prewhitened the data of the low-frequency modulations by subtracting a low-order polynomial and we computed the periodograms of the residuals. No evidence of periodicity is present either in the range 20-30 min or 90-100 min, as well as over all the range down to ~ 3 min. Most of the low-frequency noise is introduced in the periodograms by the intense X-ray flaring activity of 4U1700-37. This is the main problem to be overcome when searching for periodic modulation in the flux of a very variable source. We take the maximum value of the random noise peak-to-mean modulation as the upper limit to any true periodicity. Table 1 lists such upper limits for the ranges of period of interest and for increasing order of the polynomial

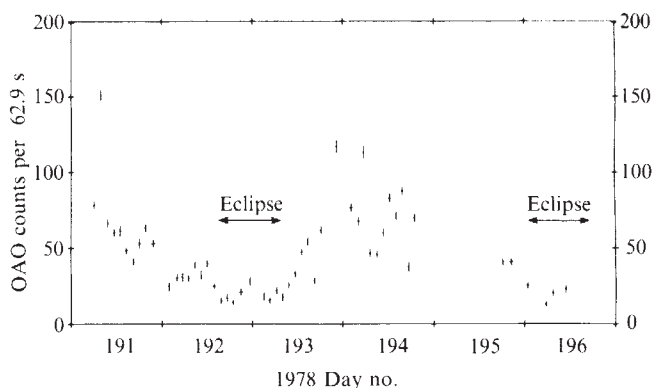


Fig. 1 The X-ray light curve of 4U1700-37, after subtraction of the diffuse sky and particle background, as observed by Copernicus is July 1978. The crosses represent the 1σ errors on the (3.1-9.4 keV) count-rate integrated in bins of ~ 1 h duration. The contamination of Sco X-2 is low (~ 20 counts per 62.9 s on average) due to the favourable orientation of the detector's field of view (the efficiency at the location of Sco X-2 was $\sim 20\%$). The times of phase 0.0 (centre of the X-ray eclipse) were calculated from the ephemeris given in ref. 2; the indicated length of the eclipse corresponds to the shortest ever observed (1975, Copernicus; ref. 2.). The gap in the data during day no. 195 is due to the observation of a different target.

fitted to the data. This was an attempt to decrease the low-frequency noise contribution to the periodograms so as to make a real, if any, periodicity show up. Table 1 shows that the technique is most useful to reduce the noise in the range of longer periods investigated. We have also searched for the suggested periodic behaviour in the 4U1700-37 X-ray light curve by folding the data modules' values in the ranges 23-25 and 96-98 min (at intervals of 0.1 min). The analysis is made difficult again by the intrinsic variability of the flux (the average peak-to-mean modulation is $\sim 20\%$ at any value of the trial period, in agreement with the results of the Fourier analysis). No significant periodicity is evident above the noise.

The fact that the most recent X-ray observation of 4U1700-37 does not show evidence of any of the proposed periodicities can be interpreted in several ways.

(1) The true period is at ~ 24 min, but it has already been shown² to be present at times and absent at others (possibly covered by the flaring activity of the X-ray source). It is, therefore, quite possible that it was again absent in the 1978 Copernicus observation. In this respect it is interesting that the mean X-ray flux of 4U1700-37 may have monotonically decreased during 1974-76 (see Table 2 in ref. 2). In the 1978 Copernicus data the X-ray flux is close to the value observed in 1975, also when no periodic modulation was detected.

Table 1 Upper limits to the peak-to-mean amplitude of any periodic modulation in the ranges of period indicated

Order of the polynomial used to prewhiten the data	Ranges of period (min)	
	20–30	90–100
1	18%	50%
3	19%	17%
6	17%	19%

(2) If a periodicity at ~ 97 min existed in March 1977³ and was rotational in origin, it is possible that it could have disappeared in the 15 months which elapsed between the 1977 SAS C and the 1978 Copernicus observations (see the current theories of accretion torques on neutron stars in binary systems^{7,8}). However, the rotation mechanism seems unlikely because of the very small probability of observing such a rapid changing period with the limited coverage of the satellites available for X-ray astronomy (the periodicity would only be present for a very short interval in the lifetime of the X-ray source). If a different origin for the proposed ~ 97 min periodicity is invoked, then it is difficult to explain how such a strong modulation (up to 60% (ref. 3)) of the X-ray flux has escaped detection in all the other Copernicus observations of 4U1700–37 (one binary cycle in July 1974 (ref. 9), two cycles in July 1975 and August 1976 (ref. 2), one cycle in July 1978). In this context, it has been suggested¹⁰ that the ~ 97 min period in the SAS C data could be spurious, arising from the way the data were collected, namely gaps in the data (due to the satellite being in the South Atlantic Anomaly or Earth blocked, or to telemetry dropouts) with the overall shape of the data set.

(3) The 'transient' X-ray periodicities reported for 4U1700–37 could be due to occasional trains of quasi-periodic events in the flaring activity of the source. This variability may be interpreted as the result of shot noise; from the periodograms obtained by Fourier analysis of the Copernicus data we deduce a characteristic exponential decay time¹¹ of ~ 15 min.

Much more observing time is required to satisfactorily clarify the temporal behaviour of 4U1700–37. In particular the data are too limited to precisely evaluate the third alternative proposed.

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X-ray observations of AM Herculis from OSO 8

THE white dwarf binary system AM Herculis (2A1815+500) has been observed in X rays at both low energies ($E \leq 10$ keV)^{1–3} and higher energies^{2,4,5}. The exact shape of the spectrum, particularly at the higher energies, has yet to be determined. We present here results from the high energy scintillation spectrometer on OSO 8. These are combined with results published elsewhere⁶ obtained concurrently with the proportional counter on the same satellite, thereby giving for the first time coincident observations of AM Her over the range 2–250 keV.

The OSO 8 instruments used to observe AM Her consist of a proportional counter⁷ covering the photon energy range 2–40 keV, and a high energy scintillation spectrometer⁸ covering the range 20–250 keV.

The source was observed during the period 1–10 October 1977. The results presented here from the scintillation spectrometer cover the whole period, whereas the proportional counter spectrum is based on 'quick-look' data taken during $\sim 20\%$ of the period. The composite OSO 8 spectrum is shown in Fig. 1. Also shown for comparison are the spectrum of Staubert *et al.*⁵ obtained on 20 September 1977, the Ariel 5 results⁴ obtained during the period 18–22 February 1977 and the OSO 8 proportional counter spectrum obtained during the period 11–12 October 1975². AM Her is an eclipsing binary system and all the results except those of Staubert *et al.*⁵ have been averaged over the whole binary cycle. Since Staubert *et al.*⁵ observed it for only part of a cycle, their results have been normalised by a factor of 0.83. This factor was obtained by comparing the phases of their observation periods with the 10–60 keV light curve of Swank *et al.*².

Variations in the spectral shape and intensity are apparent in the results up to 100 keV. Even if the Ariel 5 results⁴ in this energy range are not considered because of their low significance (~ 1.7 s.d.), there remains a statistically significant difference between the results of Staubert *et al.*⁵ and OSO 8 taken only 10–20 d apart. If a thermal bremsstrahlung spectrum, including the modified Gaunt factor, is fitted to each data set, then apparent order of magnitude changes in temperature seem to be occurring (see, for example, the temperature of ≤ 18 keV found by Staubert *et al.*⁵ to their 1977 observations and the temperature of 170 keV fitted by Swank *et al.*² to their 1975 OSO 8 data).

The X-ray emission of AM Her has already been shown⁹ to vary in soft X rays ($E \leq 10$ keV) by a factor of approximately four from one binary cycle to the next. The results summarised here indicate that changes in spectral shape also seem to be occurring. Although the changes in intensity are certainly real, the variations in temperature are not so well defined. The results from OSO 8 illustrate the confusion. In 1975 a single temperature fit to the data was found to be statistically unacceptable², whereas in 1977 the opposite was true (Swank, personal communication). Furthermore, if the spectrum of Staubert *et al.*⁵ is extrapolated to lower energies, then the flux in the 2–5 keV range would be at least one order of magnitude greater than that ever observed. Consequently, the latter results and the 1975 OSO 8 results suggest a spectral break being present, possibly at about 15 keV, on these occasions. The 1977 OSO 8 results, however, require no such feature.

If the spectrum of AM Her is definitely shown to possess a spectral break on some occasions, then at these times it will be similar to that seen from Her X-1 (ref. 10) in which a break is observed around 25 keV. The reason for the break in the Her X-1 spectrum is thought to be the modification of the Thomson cross-section in the intense (10^{13} G) magnetic field of the neutron star. The magnetic field of the white dwarf in AM Her is, however, believed to be of the order of 10^8 G from optical

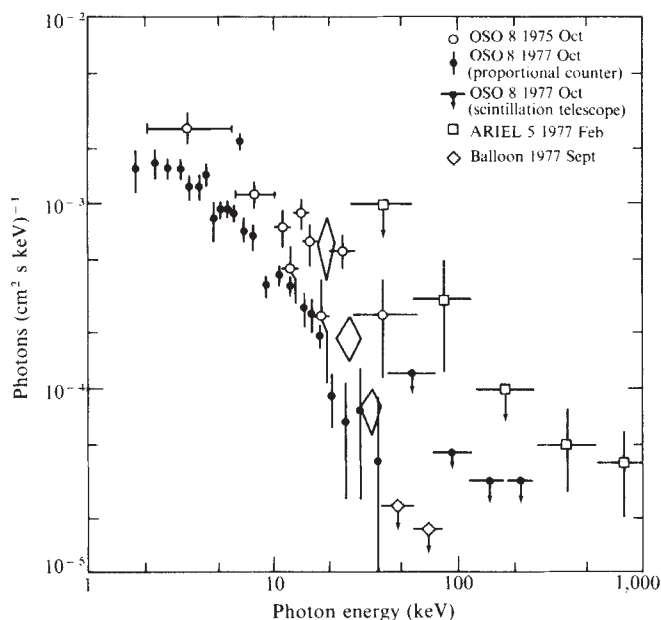


Fig. 1 Measurements of the X-ray spectrum of AM Her. The OSO 8 data below 50 keV come from the GSFC proportional counter experiment^{2,6}, while the data above 50 keV come from the present work. The Ariel 5 data is from ref. 4, and the balloon data from Staubert *et al.*⁵. Upper limits are indicated at the two standard deviation level.

polarisation measurements¹². The calculations of Canuto *et al.*¹¹ indicate that this would not be high enough to affect the X-ray photons. Consequently, if a break in the AM Her spectrum is subsequently confirmed, then some modified or alternative theory is required to explain it.

Finally, the upper limits obtained above 50 keV by Staubert *et al.*⁵ and the present work make it unlikely that a γ -ray tail, such as that seen at the 3.2 standard deviation level in the Ariel 5 data⁴, existed on these occasions. In view of the possible spectral changes suggested by the results summarised in this work, however, the existence of such a feature on other occasions cannot be excluded.

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Near IR surface brightness of southern galactic plane

NEAR IR surface of the Galaxy has been observed in balloon experiments¹⁻⁶. The stellar distribution in the inner Galaxy thus obtained is related to the distributions of H I clouds, CO clouds, γ rays, OH-IR sources and so on⁷. These observations were restricted to the northern sky, and we have recently attempted to use balloon observations from Australia to study the overall structure of the Galaxy more completely. We found that (1) humps for 2.4- μ m surface brightness are asymmetric with respect to the galactic centre and attributed to the spiral structure; (2) the 2.4- μ m galactic plane is distorted from the flat plane in the regions around the most pronounced humps; (3) IR sources are highly concentrated in the plane to compensate interstellar absorption; and (4) the central region shows a complex structure.

The instrument we used is similar to that described by Ito *et al.*². Incident radiation was focused by a 10-cm, $F1.0$ silicon lens onto 11 PbS detectors, with fields of view of 0.5°, 0.8° and 1.7° at 2.4 μ m, and 2.0° at 3.4 μ m. The whole telescope system was cooled by liquid nitrogen to reduce the thermal background noise. The optical axis was fixed at an elevation angle of 40° and regularly scanned in azimuth through a 60° range centred on the galactic plane.

The payload was launched on 25 April 1978 at the Australian Balloon Launching Station, Mildura, Victoria, Australia. The instrument functioned satisfactorily during the whole flight, and the telescope surveyed the galactic plane between $l = 290^\circ$ and 20° . During the azimuthal scans we observed several bright stars which enabled us to obtain the absolute flux value at 2.4 μ m within $\pm 10\%$, and to determine the latitude of the telescope within $\pm 0.2^\circ$. The absolute flux value at 3.4 μ m was obtained with lower accuracy.

The contour map obtained with the 1.7° field of view at 2.4 μ m is shown in Fig. 1, in which the contributions of bright stars, α -Cen, γ -Cru, and ϵ -Mus, are subtracted. The extended disk structure with about 5° width and some humps are observed. In addition, complex features are well resolved in the galactic centre region because of a low airglow level attained at a high elevation angle. Maps obtained with finer fields of view show similar features but a more pronounced structure near the galactic centre. This implies that the derived surface brightness results from an extended source and is not influenced by nearby bright stars; this can be partly confirmed using the data of the 2.2 μ m survey of stars^{8,9}.

Figure 2 shows the longitudinal distribution of the peak surface brightness near the galactic equator at 2.4 μ m observed with the 1.7° field of view. The error due to detector noise and a variation of the airglow level is as small as 3×10^{-11} W cm⁻² μ m⁻¹ sr⁻¹.

Humps at $l = -13^\circ$, -22° , -45° were first found in this experiment; similar humps at $l = 16^\circ$, 27° , 47° had been detected during previous observations. The hump at $l = 27^\circ$ indicates a strong concentration of IR sources in the region at ~ 5 kpc from the galactic centre; molecular clouds, H II regions and other young objects are also concentrated in this '5 kpc ring'⁷. However, our present result indicates that the surface brightness is distributed not symmetrically, and suggests the spiral structure of the Galaxy. Although the velocity distribution of CO clouds does not clearly show the ridge of the spiral structure¹⁰, the 5-kpc ring may represent part of the bright galactic arm.

In Fig. 2, G and S indicate the tangential directions to the galactic arms modelled from the distribution of large H II regions¹¹ and from the density wave theory mainly based on H I observations¹² respectively. The agreement is not good over the entire galactic plane, the discrepancy being larger for the southern galactic plane. However, a pair of innermost humps at

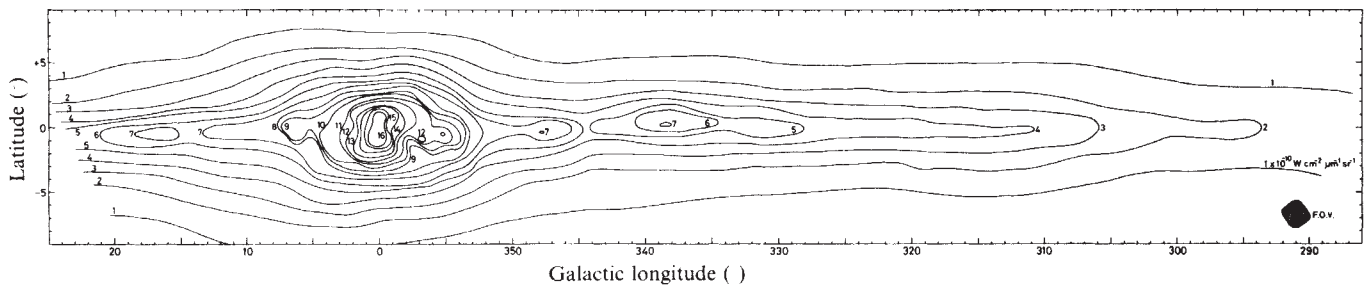


Fig. 1 The contour map of 2.4 μm surface brightness with 1.7° field of view. The shaded area indicates the field of view used.

$l = 16^\circ$ and -13° would indicate the existence of the dispersion ring. Observed asymmetry is essentially consistent with the ellipticity and the direction of the major axis in the model¹².

Figure 2*b* shows the relative colour, $[m(2.4 \mu\text{m}) - m(3.4 \mu\text{m})]$ at the surface brightness peak plotted against longitude, with the normalisation to $[m(2.4 \mu\text{m}) - m(3.4 \mu\text{m})] = 0$ at $l = 0^\circ$. A colour difference of ~ 0.3 mag is found between the disk and galactic centre directions; that is, the disk is redder than the central region. The colour difference of 0.3 mag corresponds to the excess interstellar extinction of $A_V \sim 10$ mag towards the disk direction over the galactic centre direction, consistent with the model of the distribution of interstellar dust⁷.

The latitude of the peak surface brightness for each scan is also plotted in Fig. 2*c*. Peak surface brightness is situated $\sim 0.5^\circ$ below the galactic equator for $10^\circ < l < 30^\circ$ and $\sim 0.5^\circ$ above the galactic equator for $335^\circ < l < 340^\circ$. Note that these displacements seem to be associated with bright humps at $l = 27^\circ$ and -22° . For the northern galactic plane, similar displacements are

observed for the 5 kpc ring for CO clouds¹³, H II regions¹⁴, and OH-IR sources¹⁵.

Another interesting feature is the latitude distribution of surface brightness. Even for the finest field of view at 2.4 μm , we do not find the absorbing feature which might be expected from a strong concentration of absorbing matter in the plane. This implies that the IR sources are also concentrated in the galactic plane, supporting the view that they are young objects, probably M supergiants. At 3.4 μm the galactic plane is narrower than that at 2.4 μm . This difference can be explained by weaker interstellar extinction at 3.4 μm .

The galactic centre region reveals several interesting features. First, the 2.4 μm plot within 4° from the galactic centre is asymmetric with respect to the galactic plane, similar to the tilted disk modelled from the H I observations¹⁶. Second, two features extend in longitude up to $l = 7^\circ$ and -6° . The feature at $l \sim 355^\circ$ was detected by previous observations^{3,4} as an extended IR source; its counterpart with a similar, though weaker, feature

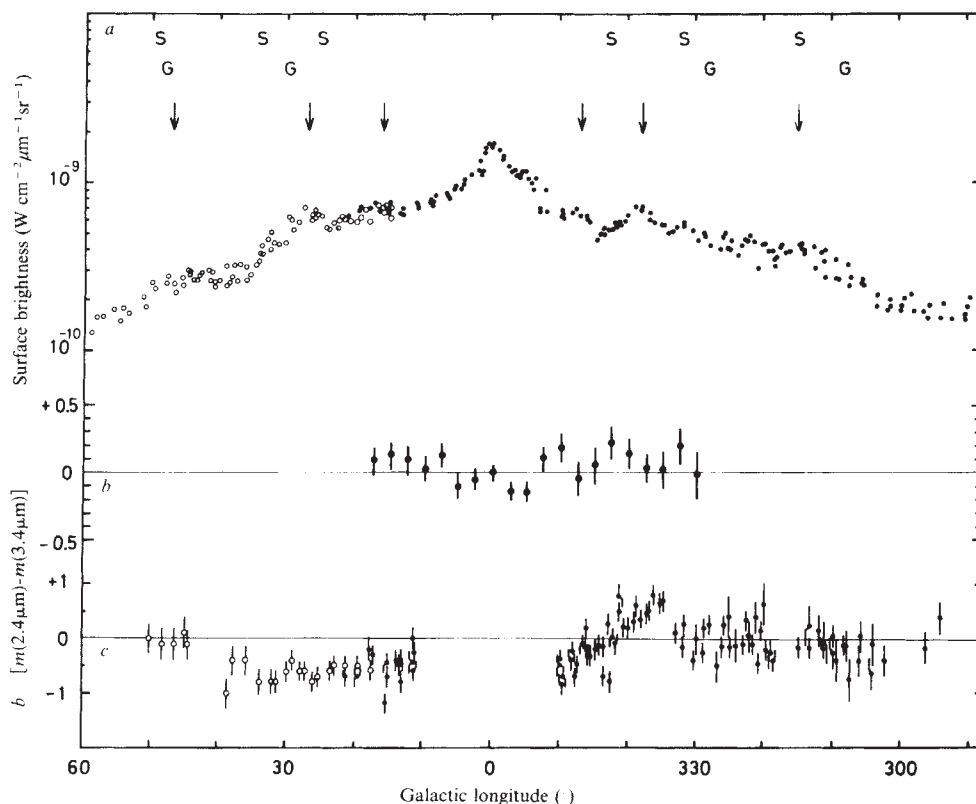


Fig. 2 The longitude distribution of the peak surface brightness at 2.4 μm for each scan with 1.7° field of view. ●, The present result; ○, our previous observations^{1,2,4}, where they have not been superseded by the present work. Arrows show the positions of characteristic humps. S and G indicate the tangential directions according to different galactic arm models. *b*, The longitude dependence of relative colour, $[m(2.4 \mu\text{m}) - m(3.4 \mu\text{m})]$, at the peak, normalised at $l = 0^\circ$. *c*, The latitude of the peak surface brightness for each scan.

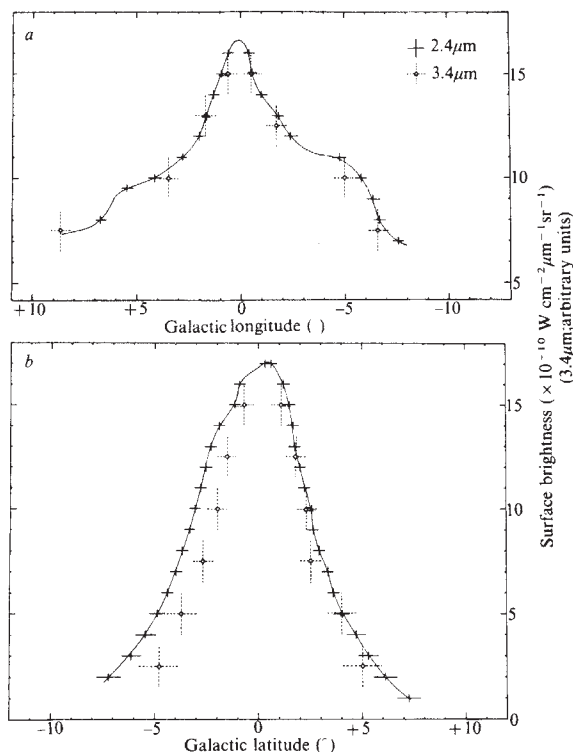


Fig. 3 *a*, The longitude distributions ($b = 0$); *b*, the latitude distributions ($l = 0$) of surface brightness at $2.4 \mu\text{m}$ and $3.4 \mu\text{m}$ on the galactic plane with 1.7° and 2° fields of view, respectively.

is first found at $l = +6^\circ$. This asymmetrical structure suggests that there exists a disk-like component on which the central bulge is superimposed. Third, there are two dark lanes intruding into the plane from below. The southern one is more pronounced and was detected by our previous observation⁴. These dark lanes may be partly explained by the dark clouds between the galactic centre and the Sun 0.5° below the $b = 0^\circ$ plane¹³. Finally, Fig. 3 compares the $2.4 \mu\text{m}$ and $3.4 \mu\text{m}$ data, and they show no appreciable colour variation with longitude, but an excess of $2.4 \mu\text{m}$ flux for $b < 0$.

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Stable 'pancake' distributions of low energy electrons in the plasma trough

WAVE-PARTICLE interactions have been recognised as the likely key to processes which control the dynamics of the Earth's magnetosphere and trigger auroral precipitation¹. The GEOS 1 and GEOS 2 satellites carry wave and particle measuring instruments which permit a full exploration of interactions occurring in the equatorial magnetosphere out to a distance of $7 R_E$ (ref. 2). The large variety of plasma wave phenomena has been reviewed by Southwood³ and Christiansen *et al.*⁴ have reported on emissions observed by GEOS 1. The geostationary position was expected to be an oasis of important wave-particle phenomena because it is in that region of the magnetosphere where cold plasma, originating from the ionosphere, mixes with hot plasma-sheet particles⁵. The large amount of data now emanating from GEOS 2 more than meets expectations, a number of relatively exotic particle distributions are being observed together with clearly associated wave spectra. Here, we report on one significant example.

The UCSD spectrometers aboard the ATS 5 and ATS 6 satellites yielded a magnificent data set for the particle environment at $6.6 R_E$ (ref. 6). This, together with data from satellites in highly eccentric orbits (such as OGO 5 and Explorer 45), has led to an established picture of particle convection, substorm injection and subsequent dispersion, excursion of the plasmopause boundary and ring current evolution.

Theories of wave generation depend on the creation of instabilities and call for anisotropic particle populations; a distribution function $f(E)$ with $df/dv_\perp > 0$ is particularly sought. The inevitable loss cone is an obvious candidate source, it can be enlarged by pitch angle diffusion⁷; also the inward convection from the tail generates thermal anisotropy ($T_\perp > T_\parallel$) but the key parameter is probably the ratio between the densities of 'cold' and 'warm' plasma components. Sufficient cold electrons are required for wave development but too many will damp the unstable mode⁸.

The suprathermal plasma analysers (SPAs) on GEOS 2 have regularly detected a population of electrons over 50 eV which are confined to pitch angles near 90° —a 'pancake' distribution. These particles can be observed near the geomagnetic equator for several hours a day but the dependence on local time is variable, as are the intensity profile and energy spectrum. GEOS also gives the first reliable measurements of cold plasma density near $6.6 R_E$ and a preliminary description of these new results is presented here. Electrostatic emissions near the half harmonics of the electron-cyclotron frequency are commonly observed near the geomagnetic equator⁹, Gough *et al.*¹⁰ present further evidence for observed correlations but a firm conclusion on cause and effect is not yet clear.

Figure 1 shows the GEOS spacecraft illustrating the position and viewing directions of the SPA sensors which incorporate simple hemispherical electrostatic analysers¹¹. The isolated boom mounting of the sensor unit with the utilisation of a bias voltage to overcome the satellite floating potential (a few volts positive) allows the collection of cold protons, the complex sheath structure obviously influences the detected fluxes but in-flight calibration is available in the plasma frequency determination from the wave experiment⁴. The conducting surface of GEOS seems to eliminate the problem of differential charging

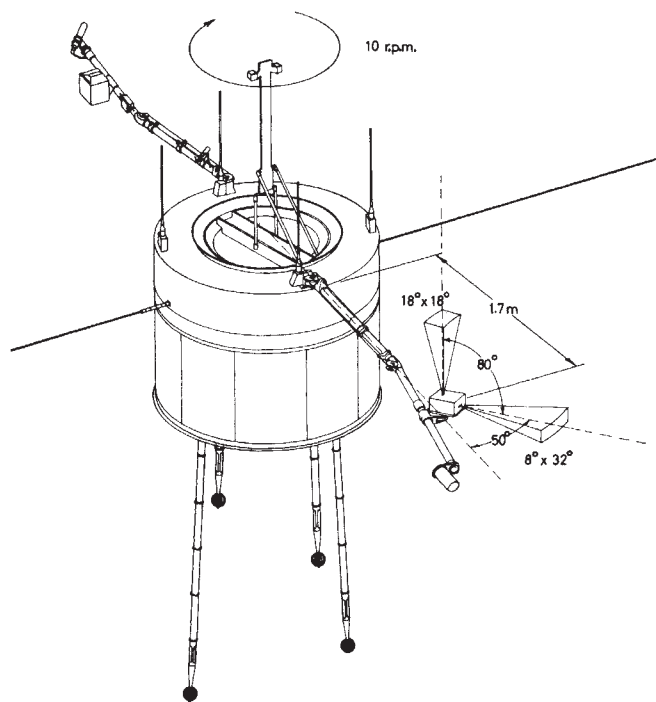


Fig. 1 GEOS satellite showing the position of the suprathermal plasma analysers.

seen on ATS¹² and this is crucial for these very low energy measurements. One analyser views parallel to the satellite spin axis but this is seldom magnetic field-aligned. In fact the axis of GEOS 2 is usually between 10° and 15° (θ_B) from the field direction and consequently the radial analyser, scanning pitch angles between $(80 - \theta_B)$ and $(80 + \theta_B)$ during a 6 s spin period, is ideally pointed to detect pancake distributions. Sixty-four energy channels ($\Delta E/E = 0.11$), logarithmically spaced, cover the full range of 0.5–500 eV but below 20 eV satellite emitted photoelectrons mask the ambient fluxes. It takes about 3 min to obtain a full coverage of energy (20–500 eV) over the available pitch angles.

Figures 2 and 3 show the energy dependence of the pancake distribution by comparing three selected pitch angles ($\alpha \leq 90^\circ$)

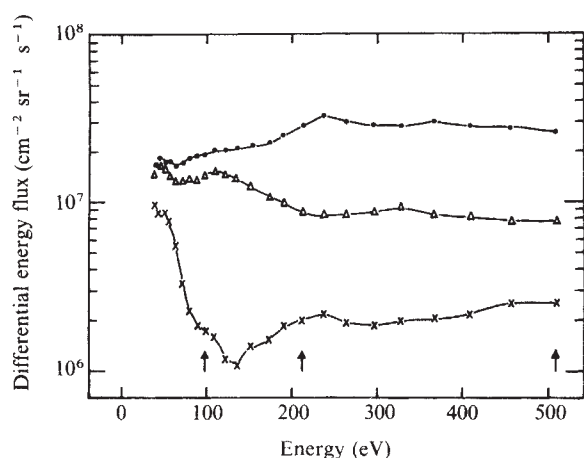


Fig. 2 Energy spectra for pitch angles: 90° (●); 69° (△); 12° (×) taken from detector viewing parallel to spin axis. (2 August 1978 at 5.05 UT; 0.75° N, 8.8° E)

and three selected energy channels. Count rates have been converted to differential energy flux and data from the parallel analyser ($\alpha = \theta_B = 12^\circ$) are included to allow interpolation to small pitch angles. Although the form of the distribution is quite variable, these curves are typical; both the peak flux and the maximum anisotropy often appear below 500 eV, the anisotropy tends to vanish at energies less than 100 eV but here the photoelectrons become significant. The degree of anisotropy can be assessed by fitting a $\sin^n \alpha$ function; where n takes a value of 10–20 at the peak the electrons are confined to pitch angles very close to 90° . The analyser acceptance angles (see Fig. 1) do produce some instrumental broadening of the feature. The

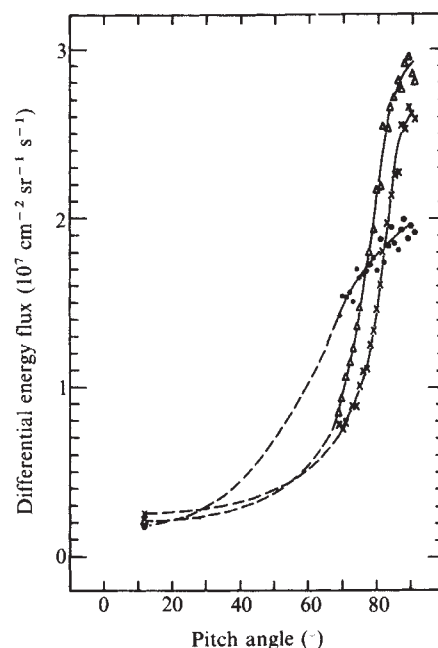


Fig. 3 Pitch angle distributions for energy channels: 211 (△); 509 (×); 98 (●) eV.

phase space density of the 90° electrons does not exhibit a positive slope when plotted against energy.

The pancake population is generally observed by GEOS 2 during the morning hours. Near local midnight the SPAs detect plasma-sheet electrons which are isotropic, the differential fluxes increase with energy but the maximum is obviously well above our 500 eV limit. Near noon or later, GEOS 2 regularly encounters a cold plasma enhancement with density N_i of 50 cm^{-3} or more. It is not always clear whether these are excursions into the plasmasphere or through detached regions. Between the two, in the morningside plasma trough, the $\alpha = 90^\circ$ electrons can persist for many hours. To search for possible correlation with electrostatic wave emission, a period of 7 days in August 1978 has been studied and Fig. 4 summarises the results. The geomagnetic planetary index K_p reached 5 on 12 August but the other days were fairly quiet. The magnetic field briefly departed from a dipolar configuration on only three occasions; V , D , H components supplied by Mariani have been used to compute $\lambda = \tan^{-1} [-V/2H]$ as an estimate of an effective magnetic latitude; this suggests that GEOS 2 was within 3° of the equator for most of the period. The cold plasma density was determined by using a sensor bias of -28 V and calibrating maximum count rates with reference to appropriate plasma frequency measurements; no attempt has yet been made to analyse the spectra but it is clear that the temperature cannot be significantly greater than 1 eV. The plasmaspheric passages tend to be earlier than would be consistent with an evening bulge

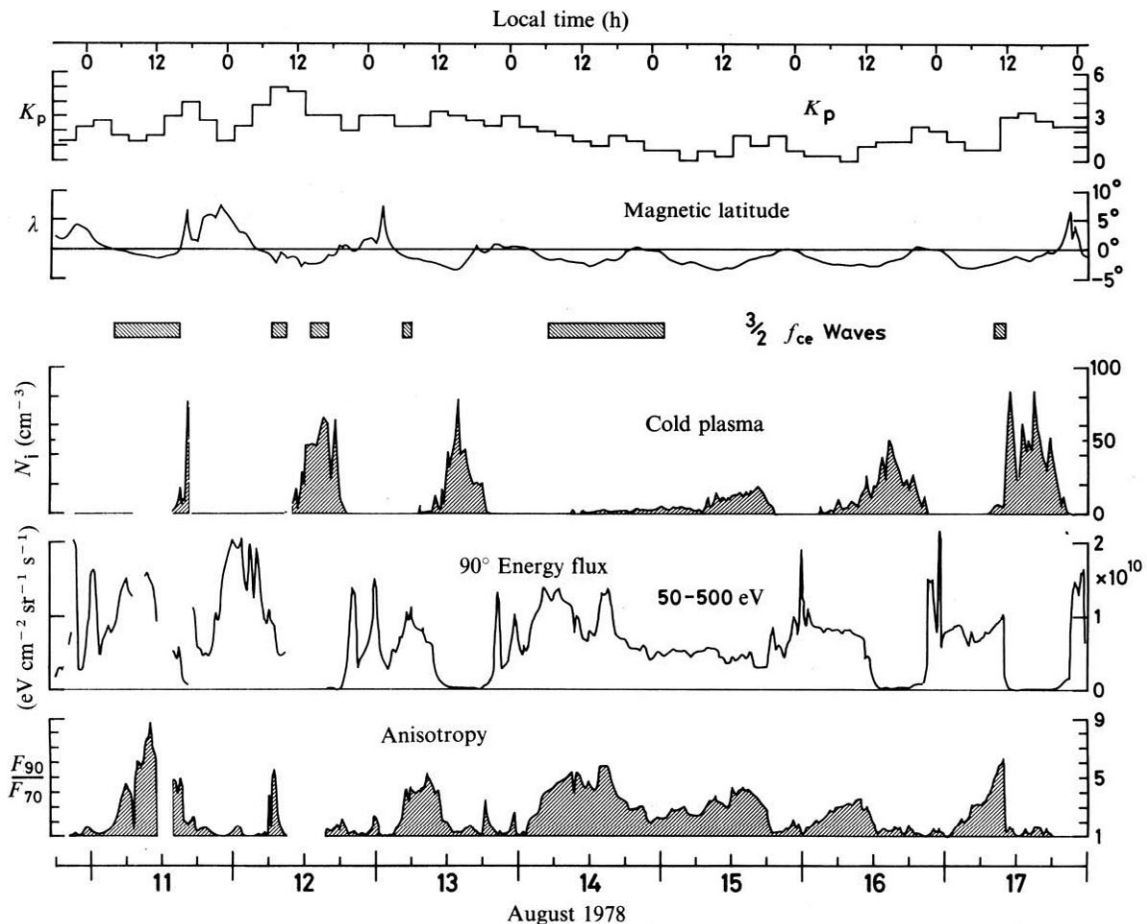


Fig. 4 One week's data from GEOS 2 showing the appearance of pancake electron distributions in relation to cold plasma enhancements and ' $3/2 f_{ce}$ ' emissions.

and N_1 can be $\sim 2 \text{ cm}^{-3}$ or more all around the orbit when K_p remains small. At such times the total energy flux for electrons between 50 and 500 eV shows that plasma-sheet interceptions can be very short or even non-existent. The flux for $\alpha = 90^\circ$ divided by that for $\alpha = 70^\circ$ provides an anisotropy index which shows the presence of the pancake distribution. The latter normally builds up during the morning hours and disappears at a plasmopause entry, there can be a sharp cut off as on 17 August but on the quiet days the pancakes persisted as N_1 did not exceed 10 cm^{-3} . The growth of the anisotropic distributions is usually from 500 eV downwards in energy and it will be interesting to examine other GEOS data (S310) to find the high energy limit.

Detection of strong electron cyclotron harmonic emissions (' $3/2 f_{ce}$ ') is indicated on Fig. 4 by the identification of times for which the integrated spectral density between f_{ce} and $2f_{ce}$ exceeds $10^{-9} \text{ (V}^2 \text{ m}^{-2})$. A high anisotropy index (>3) when combined with an absence of dense cold plasma ($<10 \text{ cm}^{-3}$) generally corresponds with strong wave generation. This striking coincidence of the pancakes and the ECH waves is a feature of all GEOS 1 and GEOS 2 data although there are a few obvious exceptions which need explanation. Gough *et al.*¹⁰ describe the complexity of the wave spectra and introduce a classification as a starting point in ordering the data; evidence of interaction is clearly identified.

We have given an early announcement of a magnetospheric particle population which must be an important quiet-time feature of the equatorial plasma trough. It has not been reported previously; GEOS 2 is the first high altitude satellite, with suitable low energy particle detectors, stationed close to the geomagnetic equator. The pancake distributions almost certainly result from wave interaction; the observed electro-

static waves seem to be responsible but the interaction is not 'local' in that the drift path of the electrons is not along the satellite orbit. The stability of the consequent distribution and the inherent equatorial confinement could then determine the morphology of the class III waves¹⁰. A detailed study is under way and we will be disappointed if this is not matched by renewed activity on the part of theoreticians.

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Interaction of electrostatic waves with warm electrons at the geomagnetic equator

WE present here observations of electron-cyclotron harmonic (ECH) waves occurring in the vicinity of the geomagnetic equator at 6.6 Earth's radii, and discuss their relevance to the morphology of electron distributions (with energies below 500 eV) observed simultaneously¹.

The observations were carried out during the slow crossing of the geomagnetic equator (4–13 August 1978) by the GEOS 2 satellite, launched into a geostationary orbit on 28 July 1978. The earlier GEOS 1 satellite, which covered a wider latitude range in a non-geostationary orbit, showed that a particular class of dayside ECH wave emissions are confined within a couple of degrees about the geomagnetic equator².

The ECH instability, characterised by emission in frequency bands between the harmonics of the electron gyrofrequency f_{ce} , has been observed in a variety of spectral forms by a number of magnetospheric satellites^{3–5}. However, because the GEOS series combines unique active plasma diagnostic techniques^{6–8} with natural wave detection⁹ the observed natural emissions can be much more readily related to local plasma parameters than can results from earlier spacecraft.

It has been shown theoretically that the ECH instability can be explained in terms of a 'basic' two-component plasma model^{10–15}: the free energy for wave amplification is provided by a hot loss-cone component ($T_h \sim 100$ eV) while a second cool component ($T_c \sim 1$ eV) presumably of ionospheric origin, aids destabilisation. For a wide range of values of the ratio of the cool to the hot electron density, N_c/N_h , the dominant instability frequency occurs close to the cool plasma upper hybrid frequency $f_{uhc} = (f_{pc}^2 + f_{ce}^2)^{1/2}$, where f_{pc} is the cool plasma frequency. Other spectral features can occupy gyroharmonic bands below and above f_{uhc} .

Because the value of the cold plasma density strongly

influences the observed spectral characteristics of the ECH waves, it has been used as a guideline for classification schemes¹⁴ including the one given below.

Note that at the geostationary orbit f_{ce} lies typically between 2 and 3 kHz, while f_{pc} spans a wide range from below f_{ce} up to above the maximum observable frequency of 77 kHz.

A spectrogram of wave data from GEOS 2 for the period covering the last hours of the 6 August and the full orbit for the 7 August 1978 (Fig. 1) shows the four main classes of ECH emissions detected.

Class I emissions, characterised by a single frequency band just above f_{ce} (typically $1.2 f_{ce}$), occur around local midnight where the spacecraft encounters the plasma sheet, dominated by hot plasma convected in from the geomagnetic tail ($f_{pc} < f_{ce}$) and can be seen about 00.00 UT and 22.00–24.00 UT on the 7 August.

Class II emissions are briefly seen as the satellite moves into the morning sector, and the cold plasma density increases. Here the dominant intensity of the emission still occupies the lowest gyroharmonic band ($f_{pc} \sim f_{ce}$) but is accompanied by weaker, higher harmonic structures.

Class III emissions, can be seen for a long period after 01.00 UT, when the cold plasma density rises ($f_{pc} > 2f_{ce}$). The dominant frequency is sometimes banded in a complex fashion, and increases to ~ 25 kHz around 19.00 UT, with accompanying $(n + \frac{1}{2})f_{ce}$ emissions between low gyroharmonics. On board plasma diagnostics show that during this period f_{uhc} is within 1 kHz of the dominant emission band.

Class IV emission¹⁰, is a weak emission in the vicinity of the f_{uhc} which is observed after 19.00 UT, when the low harmonic signals at $\sim 3/2, 5/2 f_{ce}$ disappear.

This particular orbit does not show the usual encounter with the denser evening bulge, but the typical class IV and class III emissions associated with it can just be seen during the last hours of the 6 August (extreme left of Fig. 1).

Some feeling for relative emission intensities can be gained from the plot at the bottom of Fig. 1. The upper trace is the integrated spectral density for frequencies above f_{ce} , and the lower trace is the spectral density integrated over the range from

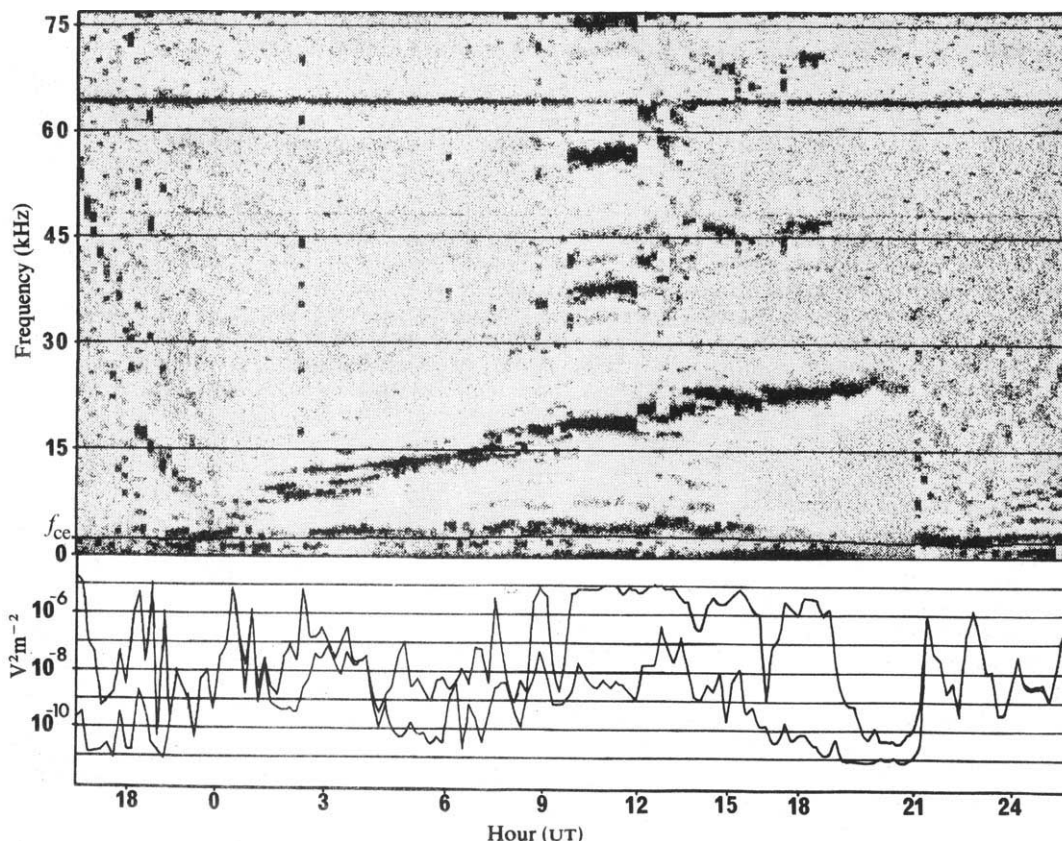
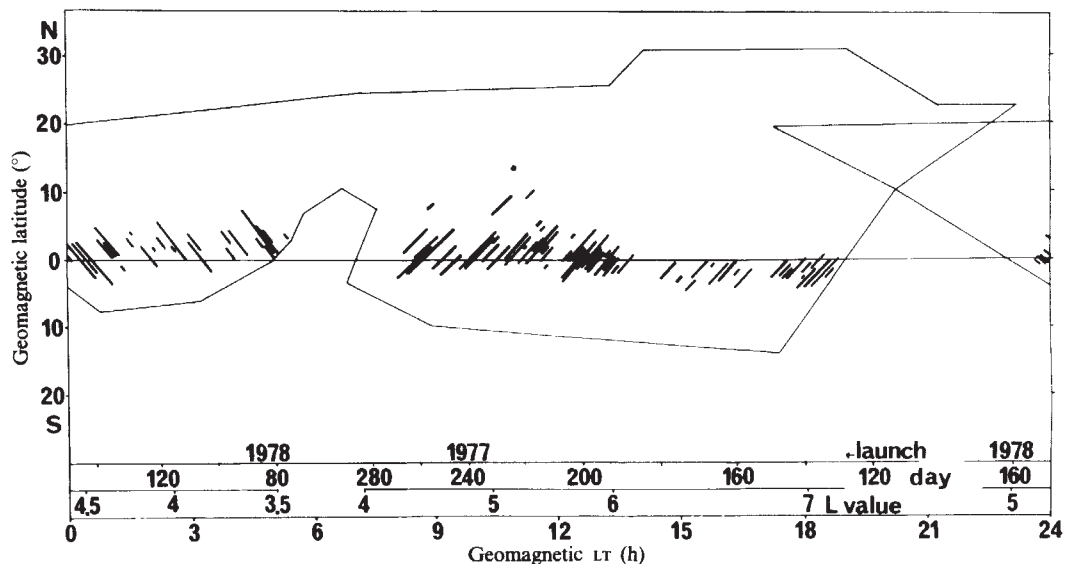


Fig. 1 Grey-scaled GEOS 2 electric field data for 7 August 1978 plotted with frequency against Universal time. The lower trace shows the integrated spectral densities for all electrostatic waves above the gyrofrequency. The lowest line is that from f_{ce} to $2f_{ce}$ only. Geomagnetic LT is UT + 1 h.

Fig. 2 All GEOS 1 observations (excluding storm periods) of type III electrostatic emissions plotted as a function of geomagnetic latitude and geomagnetic local time. Closed line is limit of observations. McIlwain L value and day number at which the satellite crossed geomagnetic equator are indicated below.



f_{ce} to $2f_{ce}$ only. Note that the spectral density peaks twice per orbit, once around midnight for class I ($E \sim 1 \text{ mV m}^{-1}$) and once in the morning-midday region for class III, when the signals are so strong ($E > 3 \text{ mV m}^{-1}$) that the wave detector saturates and harmonic generation can be observed.

The distribution of class III events (enhanced upper hybrid with $3/2 f_{ce}$ features) reported recently for dayside emissions² is shown in Figs 2 and 3, using data from GEOS 1 (perigee 2,000 km, apogee 42,000 km, $-10^\circ < \lambda_m < 30^\circ$, taking in excess of a year to survey all local times). This analysis covers 14 months of satellite operation, and class III events were recorded during the vast majority of equator crossings (Fig. 2) in which the wave analysers were in the appropriate observing modes. Those emissions which occurred far from the model field equator were accompanied by the largest differences between the field observed at the spacecraft and the modelled geomagnetic field, suggesting that in these cases the field lines were distorted.

Figure 3 further describes the equatorial confinement of the class III emissions, by showing the distribution of emission widths (Fig. 3a) and the location of the emission centres, both plotted as a function of geomagnetic latitude. Approximately 75% of the emissions were $< 4^\circ$ in latitudinal extent, and 75% were centred between $-2^\circ < \lambda_m < +3^\circ$.

In August 1978 GEOS 2 drifted slowly through the geomagnetic equator towards its present position of $\lambda_m = -3^\circ$, and during this period Wrenn, Johnson and Sojka¹ report frequent observations of remarkable electron distribution features, which show electrons in the hundreds of eV range to be confined to pitch angles within a few degrees of 90° ('pancake' distributions). Figure 4 summarises 10 d of GEOS 2 observations around the geomagnetic equator, and compares plots of hourly means of the spectral density of ECH waves, versus hourly means of the anisotropy index¹ for warm electrons with energies $< 500 \text{ eV}$. These points incorporate all data received when the two experiments (wave and particle detectors) were in the appropriate operating modes. A striking feature of Fig. 4 is that as the ECH spectral density increases there is a well defined maximum of observable anisotropy index. In particular this behaviour is strongly related to that of class III emissions (black dots) which, as pointed out above, occur mainly between 04.00 and 13.00 UT. The narrow pitch angle distributions therefore tend not to occur in the midnight sector where class I and II emissions predominate. Furthermore, from a survey of GEOS 1 data they show the same sort of equatorial confinement as class III emissions¹.

In seeking an explanation for these observations it is important to note that the combination of nightside convection of plasma sheet electrons with collisional diffusion during subsequent convection through the dayside, cannot produce

such highly pitch-angle peaked distributions. Inward convection can produce a loss-cone distribution, and therefore a source of free energy for ECH wave amplification¹⁶.

In the amplification process, resonant electrons, which satisfy the condition $\omega - k_{\parallel}v_{\parallel} = \pm n\omega_{ce}$ are scattered in velocity space, the important exceptions being those with small $v_{\parallel}$ (that is large pitch angles), which are relatively unaffected. Quasilinear theories show this clearly¹⁷ and predict among other things, that for wave fields of $\sim 1 \text{ mV m}^{-1}$ hot electrons can be rapidly scattered into the loss cone¹³.

We suggest that the 'pancake' distributions observed in conjunction with strong ECH wave activity are a remnant feature of the electron distribution, resulting from this interaction. Their evolution can be pictured as beginning with the class I emissions which are amplified by electrons freshly convected into the nightside magnetosphere, and progressing through further wave-particle interactions during the subsequent convection of the electrons around into the dayside magnetosphere. Since the pancake distributions can contribute to further destabilisation of the waves¹⁸, their presence may also be a significant factor in the equatorial confinement of class III emissions.

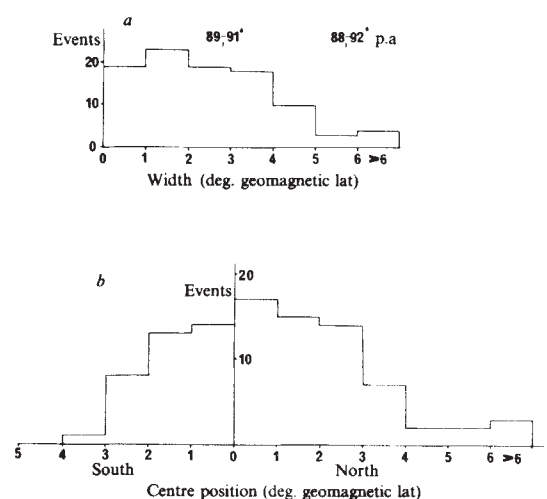


Fig. 3 a, Distribution of widths of GEOS 1 observed type III emission in degrees geomagnetic latitude. Also indicated is the equivalent equatorial pitch angle range assuming the centre of the emission is at the minimum B equator. b, Distribution of centres of type III emissions in degrees geomagnetic latitudes from field model equator²⁰.

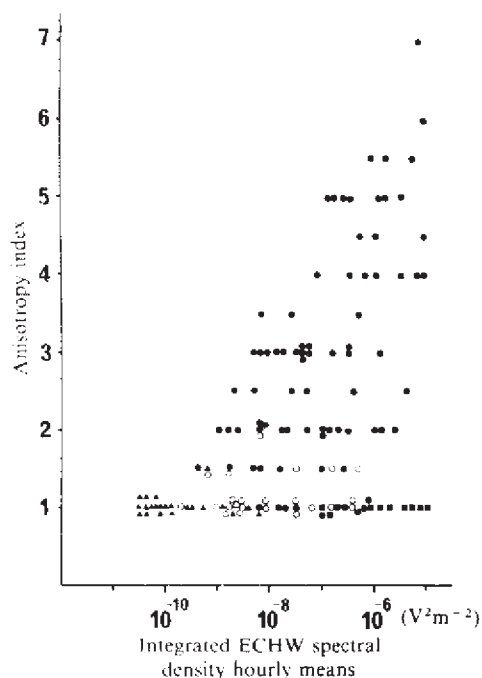


Fig. 4 GEOS 2 Hourly means of the integrated spectral density above the gyrofrequency plotted against the mean anisotropy of electrons below 500 eV between 14.44 UT on 4 August and 21.27 on 13 August. Class of emission: ○, class I and II; ●, class III; ▲, class IV; ■, class IV plasmasphere.

The role of ECH wave-particle interactions in providing the electron component of diffuse auroral precipitation has been much discussed in recent years^{3,17,19}. We hope to extend our studies into this area shortly.

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Contribution of volatile petroleum hydrocarbons to the organic carbon budget of an estuary

STUDIES on oil pollution of the marine environment have received considerable attention¹, especially work involving the effects of oil spills resulting from tanker accidents such as the Amoco Cadiz². Very little attention, however, has been devoted to assessing the importance of low level, continuous, inputs of petroleum hydrocarbons to productive estuarine environments. Investigations of hydrocarbon contamination of estuaries and rivers have concentrated mainly on the impact of non-volatile hydrocarbons³ (that is, those hydrocarbons having more than 15 carbon atoms) rather than the volatile hydrocarbons emanating from sewage discharges and industrial effluents such as petroleum refineries. We discuss here the occurrence and possible fate of these volatile hydrocarbons in estuarine systems.

During research in determining an organic carbon budget for Southampton Water estuary, the seasonal fluctuations in planktonic respiration and photosynthesis were studied. Characteristically, we observed a pronounced seasonal cycle in planktonic photosynthesis with intense activity in the mid-summer months and little in the winter. In contrast, planktonic respiration showed a less pronounced seasonal change, respiration being sustained through the winter months (Fig. 1). It has been argued that the winter respiration is supported by the external inputs (sewage, rivers and industrial effluents)⁴ and this would require a daily input with a biological oxygen demand (BOD) equivalent to 30-40 tonnes of O₂ per day. It may be seen that cumulative 5 d BOD at 15 °C is of this order (Table 1). The largest single industrial input to the estuary is the oil effluent from the Esso refinery at Fawley (the largest refinery in the UK with a refining capacity of 20 Mtonne yr⁻¹). The refinery effluent accounts for 75% of the total industrially derived carbon discharged to the estuary yearly⁴.

A detailed study has been made of the non-volatile fraction of the refinery effluent and the role and fate of these compounds in the estuarine environment⁵. These studies showed that the non-volatile fraction, the most commonly studied part of oil, could not account for the observed respiration rates. This led us to hypothesise that the volatile hydrocarbon fraction, which usually receives little attention, may play an important role in, at least, this estuarine ecosystem.

Previous work on the biodegradation of petroleum hydrocarbons has been carried out using standard microbiological techniques⁶ and it is widely accepted that marine microbial organisms can degrade oil⁷. The quantity and diversity of oil degraders present in marine systems are more likely to be higher for areas with continuous hydrocarbon discharges than pristine environments⁸. The likelihood of high numbers of petroleum utilising bacteria would be even greater for estuaries which contain high concentrations of other heterotrophs⁹. One would therefore expect that with such a large continuous input of petroleum hydrocarbons from the Fawley refinery over the past 25 yr, there would be a very significant resident population of specialised oil degraders at the refinery outfall area as well as in the estuary itself.

To investigate the degradation of the non-volatile fraction of the refinery effluent, we used large glass containers and monitored hydrocarbon concentrations using gas, solid-liquid¹⁰ and thin layer¹¹ chromatography. Quantification of compounds that were separated into broad categories of compound type was accomplished by combustion in a total carbon analyser⁵.

The average total non-volatile hydrocarbon content of the refinery effluent determined by the above method was 3 mg C per l, giving a daily input of hydrocarbons of about 1 tonne C per day. The results of our biodegradation experiments indicated that only 34% of the non-volatile hydrocarbon fraction was degraded at 15 °C over the 5 d BOD period with more

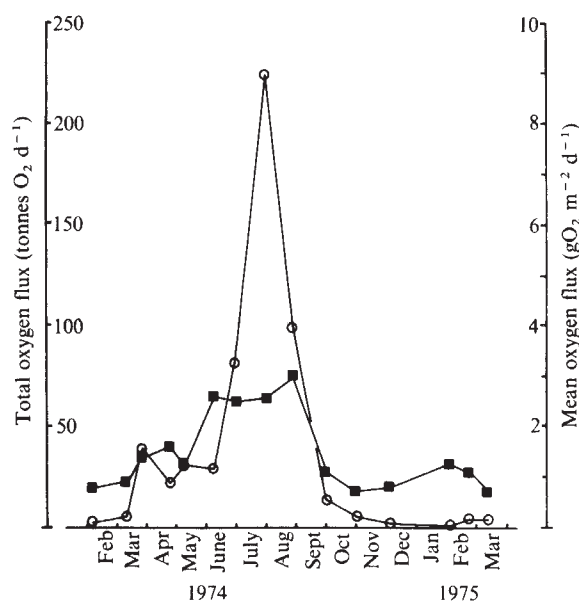


Fig. 1 Oxygen flux due to respiration and photosynthesis for Southampton Water estuary. ○, Gross photosynthesis; ■, respiration 1974–75.

than half the loss from the experimental containers attributable to evaporation processes. Assuming a respiratory quotient of 0.66 for the respiration of hydrocarbons, one may estimate the BOD stemming from the oxidation of the non-volatile fraction to be in the vicinity of 1 tonne O_2 per day. Thus it seems that the degradation of the non-volatile fraction of the refinery effluent could not account for the high BOD of 40–100 $mg O_2$ per l of refinery effluent nor could it account for the high BOD in the estuary during the winter months. Due to the possible importance of this volatile fraction, we attempted to obtain more information on the compounds in this fraction of the refinery effluent.

We analysed the refinery effluent by the standard API method 733–758 for refinery waste water used by the refinery for its monitoring programme. This involved quantification of a carbon tetrachloride extract by IR spectrophotometry at $2,930\text{ cm}^{-1}$ against a standard slop oil collected from the API gravity separators within the refinery complex. The results of these analyses indicated that the estuary received an average loading of 8–10 tonne of material per day as determined by this method. When the carbon tetrachloride extracts were evaporated at 40°C under vacuum and then redissolved in carbon tetrachloride and measured as before, the levels returned to 1–2 tonne per day. This was consistent with the results determined by the TLC/combustion method described previously. Gas chromatograms of the pre-evaporated and evaporated fraction are given (Fig. 2). Much of the extremely volatile material (hydrocarbons having 5 or less carbon atoms) is masked by the large solvent front at the start of the temperature programme. Ultraviolet (UV) analysis at 254 nm against the same standard

indicated that the removal of UV absorbing material (mainly aromatics and olefins) was similar in magnitude to those compounds absorbing at the $2,930\text{ cm}^{-1}$ region of the IR. This tentatively indicated that the refinery effluent contained both aliphatic and aromatic compounds in the volatile fraction.

In an attempt to characterise the volatile fraction in more detail we used a cryogenic trapping system¹². Volatile compounds were trapped in loops cooled by solid CO_2 and analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). This yielded a wide range of volatile *n*-alkane and aromatic hydrocarbons. Aromatics such as benzene, toluene and xylene were present in concentrations of 120, 211 and $562\text{ }\mu\text{g l}^{-1}$ respectively, indicating the importance of these compounds to the whole refinery input.

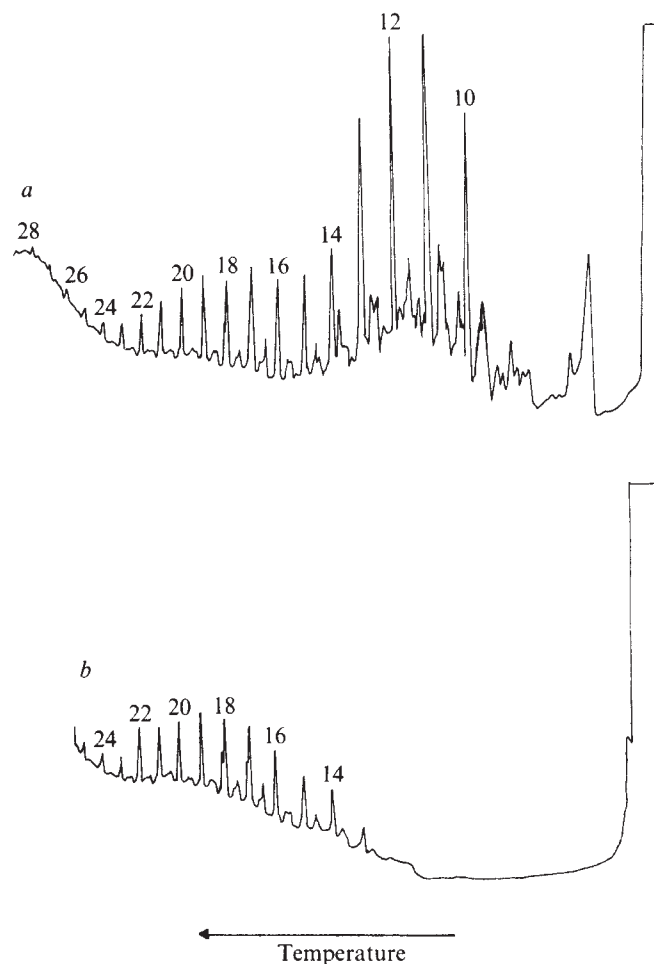


Fig. 2 Gas chromatograms of *a*, the total (volatile + non-volatile) compared with *b*, the non-volatile hydrocarbon composition of refinery effluent.

One would expect the residence time of these compounds in the estuarine environment to be similar to that of oceanic environments, aside from the obvious possibility of rapid biodegradation due to increased microbiological activity. For example, orders of magnitude changes in water concentrations of ethane, propane, butane and pentane were found over baseline in the proximity of an oil slick¹³. Sackett and Brooks also report volatile hydrocarbons present 8 km from an underwater drilling rig; concentrations of ethane alone were as high as 0.3 mg l^{-1} .

The solution mechanism of these volatile hydrocarbons is dependent on the solubilities of the compounds in water relative to their vapour pressures. Also, as molecular weights increase more material is partitioned into the gas phase¹⁴. With petroleum hydrocarbons, partitioning into the gas phase decreases in the order of *n*-alkanes, olefins, cyclo-alkanes and

Table 1 Sources of organic material in Southampton Water estimated for 1974¹⁷

	Annual input (tonne C yr^{-1})	Percentage of total annual organic input	Daily winter BOD
Phytoplankton	3,000	23%	<5.0
Rivers	1,400	11%	4.2
Sewage	1,580	12%	3.2
Industry	6,800	53%	37.0
(Petroleum 75%)		(Petroleum 40%)	

River input sampled upstream of major sewage and industrial outfalls.

aromatics¹⁵. McAuliffe¹⁵ states that these volatile hydrocarbons remaining in the water column are eventually removed through biodegradation; we feel that this is also the fate of volatile hydrocarbons in Southampton Water.

In conclusion, the data that we have accumulated during 3 years on Southampton Water estuary suggest that the volatile compounds found in industrial effluents play a significant part in the whole carbon balance of the estuarine ecosystem. The importance of volatile fractions of petroleum hydrocarbons with regard to industrial discharges to estuaries has, to some extent, been overlooked in the past. There is evidence that these hydrocarbons, especially the aromatic component, are toxic to marine life¹⁶. These considerations indicate the need for more work in valuable estuarine areas to understand the occurrence, fate and impact of these volatile compounds.

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Transformation of goethite to maghaemite in CsI disks

THE formation and stability of γ -Fe₂O₃ (maghaemite) is still a puzzle, as this oxide is metastable with respect to its isomorphous form α -Fe₂O₃ (haematite). However, there are increasing reports about its natural occurrence in certain soils and sediments (see ref. 1 and refs therein). Maghaemite, like magnetite, Fe₃O₄, is ferrimagnetic and its artificial occurrence may therefore be important in carrying out magnetic concentrations in various processes of iron ores industry²; as well as finding widespread application in the electronic industry³. The artificial preparation of maghaemite is usually carried out by low temperature dehydration of lepidocrocite⁴, γ -FeOOH, or careful oxidation of powdered magnetite^{5,6}.

Goethite, α -FeOOH, heated in the presence of organic matter to 300°C, may also be transformed into maghaemite^{7,8}. The optimal conditions for obtaining maghaemite in soils is by heating them in nitrogen, then in air at about 550 °C, which is the

Table 1 Widths at half heights (in 2 θ units) of characteristic X-ray diffraction peaks of α -Fe₂O₃

hkl	d (Å)	Haematite	Protohaematite (a)*	Protohaematite (b)†
		NT	PT	ET
012	3.688	0.24	0.47	0.63
104	2.703	0.24	0.47	0.71
110	2.518	0.27	0.31	0.39
113	2.209	0.27	0.39	0.47

NT, not transformed to maghaemite; PT, partly transformed to maghaemite; ET, easily transformed to maghaemite.

* Protohaematite (a) is obtained by heating goethite powder to 450 °C.

† Protohaematite (b) is obtained by heating goethite in CsI disk to 450 °C.

temperature at which the organic matter most readily produces a reducing environment⁹. When heating the soils in air the formation of maghaemite is negligible. The mechanism suggested for the last reactions in soil, is as follows¹: gases such as carbon monoxide, produced by the combustion of soil organic matter, reduces finely divided iron oxides and hydroxides to magnetite and on cooling the latter may be subsequently oxidised to maghaemite when air enters the matrix. Normally, thermal treatment of goethite at temperatures 200-600 °C in the absence of organic matter results in a poorly crystalline form of α -Fe₂O₃ which is defined here as protohaematite and which at higher temperatures is converted into the well ordered crystalline variety of α -Fe₂O₃, known as haematite. We show here that maghaemite can be obtained by heating goethite or protohaematite in CsI disks in the absence of organic matter. IR spectroscopy has been previously used successfully to differentiate unambiguously between magnetite, maghaemite and haematite³, as well as between protohaematite and haematite¹⁰.

In the present study the transformation of synthetic goethite to maghaemite in CsI disks was followed by IR absorption spectroscopy and supported by magnetic evidence. Disks of 13 mm diameter were prepared by hand grinding 0.5-1.5 mg synthetic goethite in 400 mg CsI and pressing at 30 ton for 30 s. For the reaction to occur, every disk must be reground and re-pressed at least three times. The optimal conditions were: heating the disks to 250 °C for one night and then at 500 °C during several nights. Alternatively, the disks were directly heated to 500 °C, but in the latter case the results were less satisfactory. Each day the disks were re-pressed and IR spectra were recorded at ambient temperature using a Perkin-Elmer 283 IR spectrophotometer. Some representative IR spectra are shown in Fig. 1.

The spectrum obtained after heating the goethite disk for one night at 250°C is characteristic of protohaematite (Fig. 1a). This indicates that at the initial stage, goethite is transformed to protohaematite at this temperature. When the same disk is then heated at 500 °C during one day (24 h), a different spectrum results, presumably corresponding to a transitional oxide (Fig. 1b). This spectrum does not resemble a spectrum of magnetite³ nor that of wustite¹¹. On further heating of the disk at 500 °C this transitional oxide is transformed to maghaemite, as inferred from the corresponding IR spectrum (Fig. 1c). Additional evidence for this transformation is that the disk is attracted by a magnet, whereas disks of goethite in CsI or of pure CsI do not show magnetic activity in the presence of the same magnet.

Unsuccessful attempts were made to obtain maghaemite: (1) from more concentrated CsI disks of goethite; (2) from disks which were not reground and repressed for several times even after long grinding periods (up to 30 min); (3) from powders resulting after the third repressing and fourth regrinding of the disks, when the powders were consecutively heated to 250 and 500 °C; (4) if the temperature of the second stage heating was below 500 °C. In (1), (2) and (3) protohaematite was the main product of the thermal treatment; in (4) the transitional oxide was the main product at >400 °C.

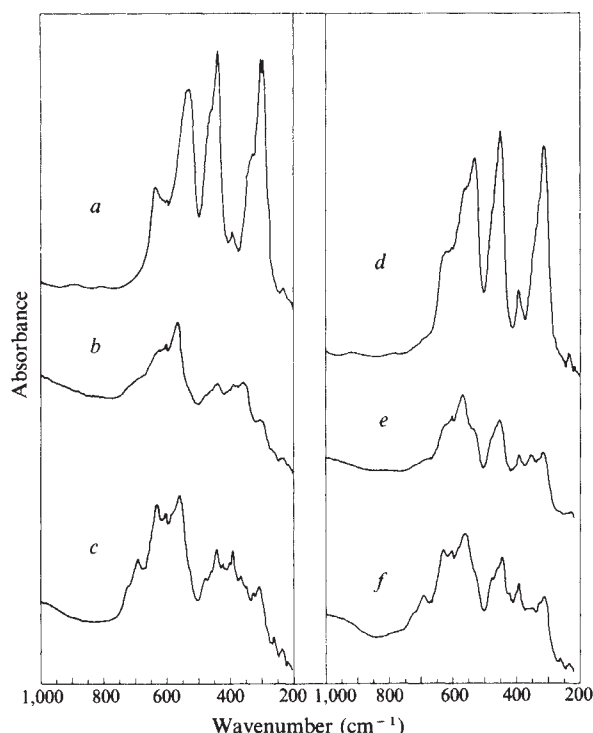


Fig. 1 IR spectra of iron oxides in CsI disks: *a*, protohaematite obtained by heating goethite disk one day at 250 °C; *b*, transition iron oxide obtained by heating disk *a* 1 d at 500 °C; *c*, maghaemite obtained by heating disk *a* 4 d at 500 °C; *d*, protohaematite obtained by heating goethite powder 5 d at 450 °C; *e*, transition iron oxide obtained by heating disk *d* 1 d at 500 °C; *f* maghaemite obtained by heating disk *d* 14 d at 500 °C. (*f* also contains some of the transition iron oxide; *e* and *f* contain small amounts of protohaematite).

The transformation of protohaematite (obtained by heating goethite powder at 450 °C for 5 days) to maghaemite in CsI disks was also followed by IR spectroscopy (Fig. 1*d, e, f*). Here again, the optimal conditions for obtaining maghaemite are a small oxide concentration, regrinding and re-pressing the disk for at least three times and heating the disk to 500 °C as a whole and not as powder.

As with goethite the transformations of protohaematite into maghaemite in CsI disk also occurs through a transitional oxide. However, bigger amounts of protohaematite remain untransformed after one day heating at 500 °C, compared with the amounts of protohaematite which are found in the goethite disk heated in the same conditions. Moreover, when protohaematite is the starting material the reaction rates are slower. The reaction was completed after less than four days when starting with goethite, but was far from completion even after 14 days when starting with protohaematite (compare Fig. 1*c* and *f*).

The thermal transformations of haematite (obtained by heating goethite powder at 1,000 °C) and of natural Al-bearing goethite (from Venezuelan laterites¹²) to maghaemite in CsI disks were unsuccessful in the present experimental conditions.

The protohaematite → maghaemite transformation is an epitaxial process in which accord between the initial and resultant lattices occurs in two dimensions. Protohaematite has a hexagonal close-packed oxygen sublattice whereas maghaemite has a cubic one. The ordering of layers shifts from *ababab* in protohaematite to *abcbcb* in maghaemite. One would expect that such a shift might occur when the *ababab* association is highly defective and/or the crystal particles are very small, as in the case of protohaematite but not of haematite.

The crystal defects, the degree of crystallinity and the small size of the protohaematite particles cause the X-ray diffraction

peaks to broaden. Protohaematite was prepared by heating gradually to 450 °C a disk composed of 150 mg synthetic goethite and 300 mg CsI (the mixture being ground for 30 min before pressing). Table 1 summarises widths of half heights of the characteristic diffraction peaks showing that there is an inverse correlation between the widths of X-ray diffraction peaks of α -Fe₂O₃ and the ease of forming maghaemite. As may be expected from thermodynamic considerations haematite with the highest degree of crystallinity fails to transform into maghaemite, whereas protohaematite formed in a CsI disk and which has the lowest degree of crystallinity, is most easily transformed into maghaemite.

The present study does not enable us to draw a firm conclusion on the stage at which the crystal nuclei of maghaemite are obtained. However, we can suggest that the nucleation of maghaemite occurs in the CsI/Fe₂O₃ interface because: small particle-size is not sufficient for the reaction to occur; the importance of regrinding and re-pressing; the reaction does not take place in a powdered medium; and other alkali halides (including KI) fail to cause maghaemite to form from goethite or from protohaematite with the same treatments.

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Sequencing of the 3'-terminal region of a 16S rRNA gene from *Zea mays* chloroplast reveals homology with *E. coli* 16S rRNA

CHLOROPLASTS are semi-autonomous organelles which possess their own DNA coding for organelle-specific RNAs and proteins. The translation process within a chloroplast is mediated by its own ribosomes of the 70S type, which are different from the 80S ribosomes of the cytoplasm. In view of this key function of 70S ribosomes for the organelle-specific protein synthesis we have undertaken to analyse rDNA from *Zea mays* chloroplasts at the nucleotide level, as we considered that this should allow a deduction of rRNA primary structures, and analysis of their mode of function and evolutionary relationship. DNA from *Zea mays* chloroplasts is a circular molecule of about 135 kilobase pairs, on which two rRNA coding regions are positioned in opposite orientation within two 22-kilobase pair

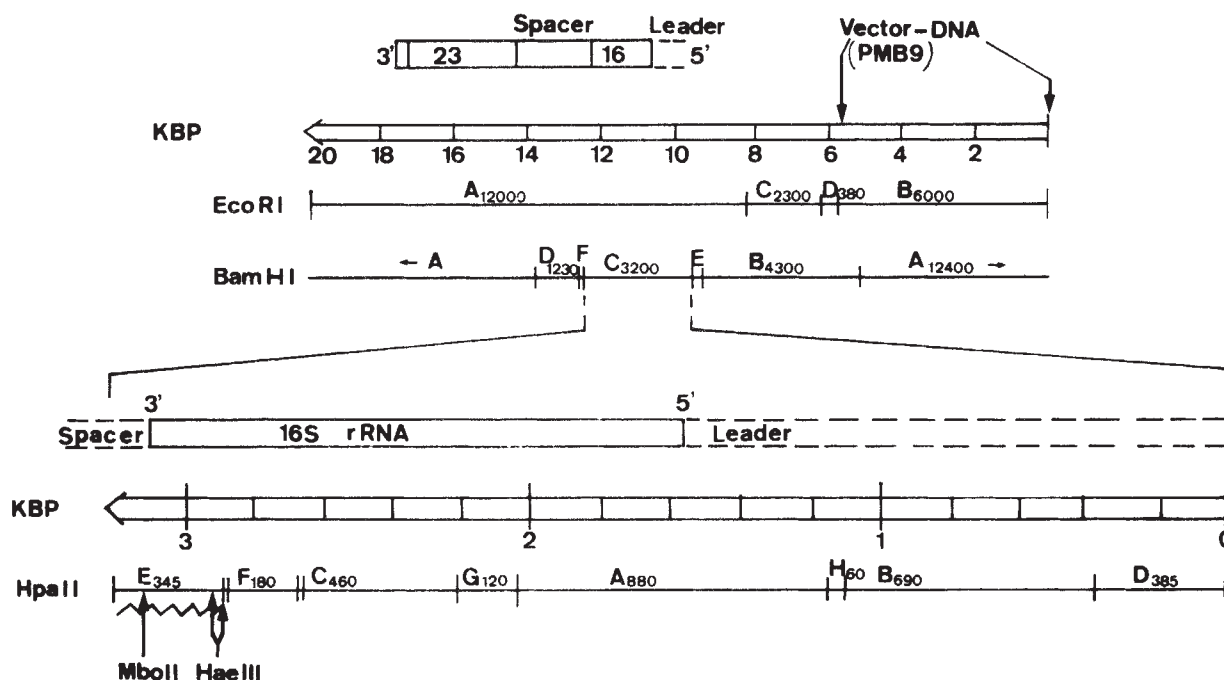


Fig. 1 Position of rRNA genes and of fragment *Bam*HI-*C/Hpa*II-*E*₃₄₅ (wavy line) on the physical map of plasmid pZmc134. Subscript numbers of the fragments refer to their approximate size in base pairs.

inverted repeats¹. Each region includes one copy of a 16S rRNA, a 23S rRNA and a 5S rRNA gene and a spacer between the 16S and 23S rRNA genes. One such rDNA region has been linked to the plasmid vector pMB9 within the *Escherichia coli* clone pZmc134 (ref. 1), and this clone was therefore used for isolation, mapping and sequencing of the respective DNA fragments (Fig. 1)². We present here the sequence coding for the 3'-terminal part of chloroplast 16S rRNA and for the adjacent part of the spacer region, and report that there is extensive homology between this region of 16S rRNA and the 16S rRNA gene from *E. coli*.

Hybridisation^{1,2} and fine mapping² data indicated that the region coding for the 3' terminus of plastid 16S rRNA is likely to be positioned at one end of fragment *Bam*I-*C*, which, after further cleavage with the restriction enzyme *Hpa*II, produces the subfragment *Bam*I-*C/Hpa*II-*E*₃₄₅ (Fig. 1). This fragment, characterised by a *Bam*I site at one end and a *Hpa*II site at the other end (Fig. 2), was isolated and used for sequence analysis according to the dimethylsulphate/hydrazine procedure³ with minor modifications as described⁴. After terminal labelling of this fragment the two labelled ends were separated by cleavage with *Hae*III or *Mbo*II (for position of cleavage sites see Figs 1,

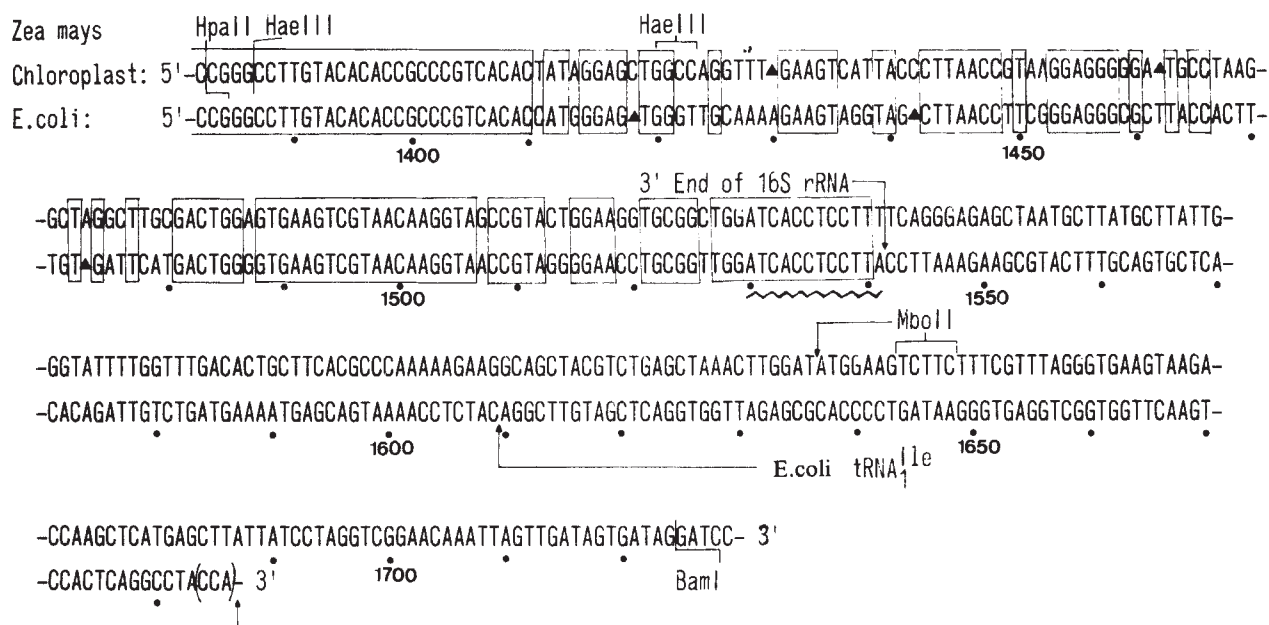


Fig. 2 3'-terminal portions of 16S rRNA genes from *Zea mays* chloroplasts (top row in each line, identical to the nucleotide sequence of fragment *Bam*I-*C/Hpa*II-*E*₃₄₅) and from *E. coli*^{5,6,9,17} (bottom row in each line). The sequences shown are of only one DNA strand, which is the non-coding, RNA-like strand. It should be noted that the sequence of the maize fragment *Bam*I-*C/Hpa*II-*E*₃₄₅ is depicted in opposite polarity to that in Fig. 1. Numbering refers to *E. coli* 16S rDNA^{5,9}. The *E. coli* spacer part containing the tRNA^{ile} gene is deduced from the tRNA^{ile} primary structure⁷. Homologous sequences are marked by boxes. The sequences at the 3' end complementary to initiator regions of prokaryotic mRNAs^{7,10,11} are underlined by wavy lines. Triangles within sequences symbolise deleted positions compared with the other DNA.

Accumulation of an mRNA and protein in interferon-treated Ehrlich ascites tumour cells

INTERFERONS are glycoproteins which are synthesised by various vertebrate cells in response to virus infection or some other stimuli. They are secreted and then interact with other cells and convert these into an antiviral state, in which the multiplication of viruses is impaired¹. It is thought that transcription and translation are required for the establishment of the antiviral state as actinomycin D or inhibitors of protein synthesis, suitably administered, prevent or delay the effect^{2,3}. Results with enucleated cells also support this idea⁴. We show here that treatment of Ehrlich ascites tumour (EAT) cells with highly purified interferon results in the accumulation of a particular mRNA and the corresponding protein within the cells. However, we have not yet identified the function of the induced protein.

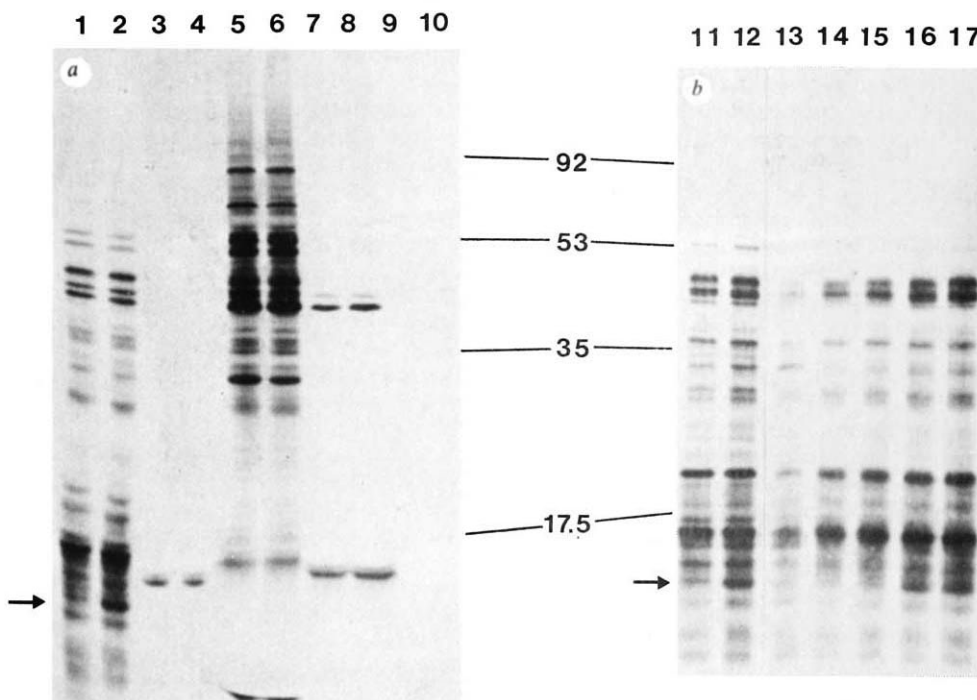
Messenger RNA was prepared from monolayers of EAT cells treated with 500 U ml⁻¹ interferon for 8 h and from control cells and was translated both in the wheat germ system and in the nuclease-treated reticulocyte lysate. The radioactive translation products were analysed by SDS gel electrophoresis (Fig. 1). In the wheat germ translation system (Fig. 1, tracks 1, 2), compared with the mRNA from control cells, the mRNA from interferon-treated cells yields an increased amount of one protein. This protein (Fig. 1, arrow) migrates with a molecular weight of

14,500. Translation of mRNA from cells which have been simultaneously treated with interferon and actinomycin D results in only the control level of the 14,500-MW protein (Fig. 1, track 13). The translation products in the reticulocyte lysate (Fig. 1, tracks 5, 6) extend to a higher molecular weight than those in the wheat germ system but the 14,500-MW region of the gel is obscured by the large amount of non-radioactive globin in the reticulocyte lysate.

To show that the increased production of the 14,500-MW protein was not a consequence of differential post-translational processing, we first translated in the presence of radioactive amino acids separately the mRNA from control cells and the mRNA from interferon-treated cells. Each of these two mixtures was then further incubated with the products obtained by translating mRNA from either control or interferon-treated cells in the presence of non-radioactive amino acids. The second incubations were carried out in the presence of anisomycin to prevent further protein synthesis. There is neither degradation of the radioactive 14,500-MW protein when it is mixed with the non-radioactive translation products of control mRNA (Fig. 1, track 16), nor production of the 14,500-MW protein when the radioactive translation products of control mRNA are mixed with the non-radioactive translation products of the mRNA from the interferon-treated cells (Fig. 1, track 15). These results make it highly likely that treatment of the cells with interferon results in increased accumulation of the mRNA coding for the 14,500-MW protein.

To compare the *in vitro* translation products of mRNA from control cells and mRNA from interferon-treated cells more closely, we analysed them by two dimensional (2D) gel

Fig. 1 Mouse EAT cells were grown in monolayers in GIBCO F15 medium containing 7.5% fetal calf serum. Mouse interferon ($0.5-1 \times 10^9$ U mg⁻¹) was prepared from EAT cells essentially as described previously¹¹. Monolayers of EAT cells were treated as indicated with 500 U ml⁻¹ interferon for 7.5 h, or with 500 U ml⁻¹ interferon and 2 µg ml⁻¹ actinomycin D for 7.5 h or kept as controls and then collected into phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.2) by scraping with a rubber policeman. The cells were washed twice with PBS, frozen, thawed and extracted twice with 0.15 M NaCl, 10 mM Tris-Cl pH 7.5, 1 mM MgCl₂, 0.075% Triton X-100. After digestion with 0.3 mg ml⁻¹ proteinase-K in the presence of 0.5% SDS and 10 mM EDTA at 37 °C for 10 min, total cytoplasmic RNA was extracted by the phenol/chloroform/isoamyl alcohol method¹². This RNA was fractionated on columns of oligo(dT)-cellulose (Collaborative T2) by application in 0.5 M NaCl, 10 mM Tris-Cl pH 7.5, 0.1% SDS and elution with 10 mM Tris-Cl pH 7.5, 0.1% SDS¹³. After recovery by ethanol precipitation, the unbound [poly(A)⁻] and the bound [poly(A)⁺] fractions were dissolved in water and stored at -70 °C. Eight 150 mm tissue culture dishes of 50% confluent control and interferon-treated cells typically yielded 2.05 mg poly(A)⁻ RNA and 0.12 mg poly(A)⁺ RNA. A similar quantity of cells treated with interferon and actinomycin D gave 1.57 mg poly(A)⁻ RNA and 0.04 mg poly(A)⁺ RNA. *In vitro* translations were in the wheat germ system¹⁴ or mRNA-dependent reticulocyte lysate⁶ with 150 µCi ml⁻¹ of a mixture of ³⁵S-methionine and cysteine as radioactive label. Poly(A)⁻ RNA was translated at 500 µg ml⁻¹ and poly(A)⁺ RNA at 60 µg ml⁻¹ except in track 13 where poly(A)⁺ RNA was at 20 µg ml⁻¹, reflecting the lower amount of RNA in the actinomycin D-treated cells. After translation at 30 °C for 60 min, aliquots were analysed by SDS gel electrophoresis⁵ and fluorographs^{15,16} are shown. *a*, Tracks 1, 2, 5, 6 poly(A)⁺ RNA, 3, 4, 7, 8 poly(A)⁻ RNA, 9, 10 no mRNA was translated in 1, 2, 3, 4, 9 the wheat germ system or 5, 6, 7, 8 the reticulocyte lysate. Tracks 1, 3, 5, 7 are RNA from control cells, 2, 4, 6, 8 RNA from interferon-treated cells. *b*, Translation in wheat germ system of poly(A)⁺ RNA from control cells (11), interferon-treated cells (12), cells treated with interferon and actinomycin D (13). Tracks 14-17: poly(A)⁺ RNA from control or interferon-treated cells was translated for 60 min in the wheat germ system in the presence of ³⁵S-methionine and cysteine and then an aliquot of each was mixed with an equal volume of duplicate translations containing either control or interferon-treated cell poly(A)⁻ RNA and no radioactive label. These reactions were then further incubated at 30 °C for 45 min in the presence of 0.2 mM anisomycin. Tracks 14, 15 first (labelled) incubation control cell mRNA; tracks 16, 17 first (labelled) incubation interferon treated mRNA; tracks 14, 16 mixed with control cell mRNA translation products; tracks 15, 17 mixed with interferon-treated cell mRNA translation products. The experiments in (*b*) used independent preparations of mRNAs and wheat germ system from those in (*a*). The vertical scale shows the positions to which standard proteins (molecular weights in kilodaltons) migrated on the gel.



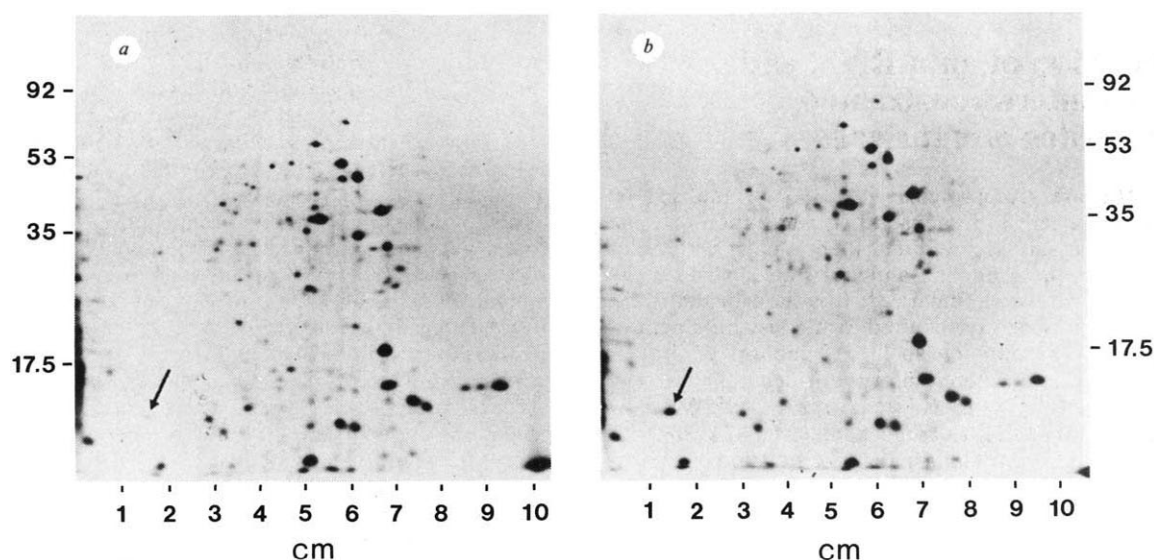


Fig. 2 Poly(A)⁺ RNA from *a*, control cells; *b*, cells treated with 500 U ml⁻¹ interferon for 8 h was translated at 60 µg ml⁻¹ for 60 min in the wheat germ system with ³⁵S-methionine and cysteine as radioactive label. Samples were then made up to 9 M urea, 2% ampholines (pH 3–10, Bio-Rad), 1% NP-40, 2.5% 2-mercaptoethanol and analysed by 2D gel electrophoresis as described by O'Farrell⁵ except that the isoelectric focusing gels contained 2% Bio-Rad ampholines (pH 3–10) and the second dimension was on non-gradient 15% gels. The figure shows fluorographs of the gels. The equivalence of cm and pH is given in Fig. 3 and the vertical scale shows the positions to which standard proteins (molecular weights given in kilodaltons) migrated on the gel.

electrophoresis⁵ (Fig. 2). In this system the 14,500-MW induced protein (arrowed) shows up clearly at pI 7.5. We cannot yet find any other significant difference between the *in vitro* translation products of the two mRNA preparations.

The experiments shown in Fig. 3 were designed to find whether the elevated level of mRNA coding for the 14,500-MW protein is manifested in an increased amount of this protein in interferon-treated cells. EAT cells were treated with interferon

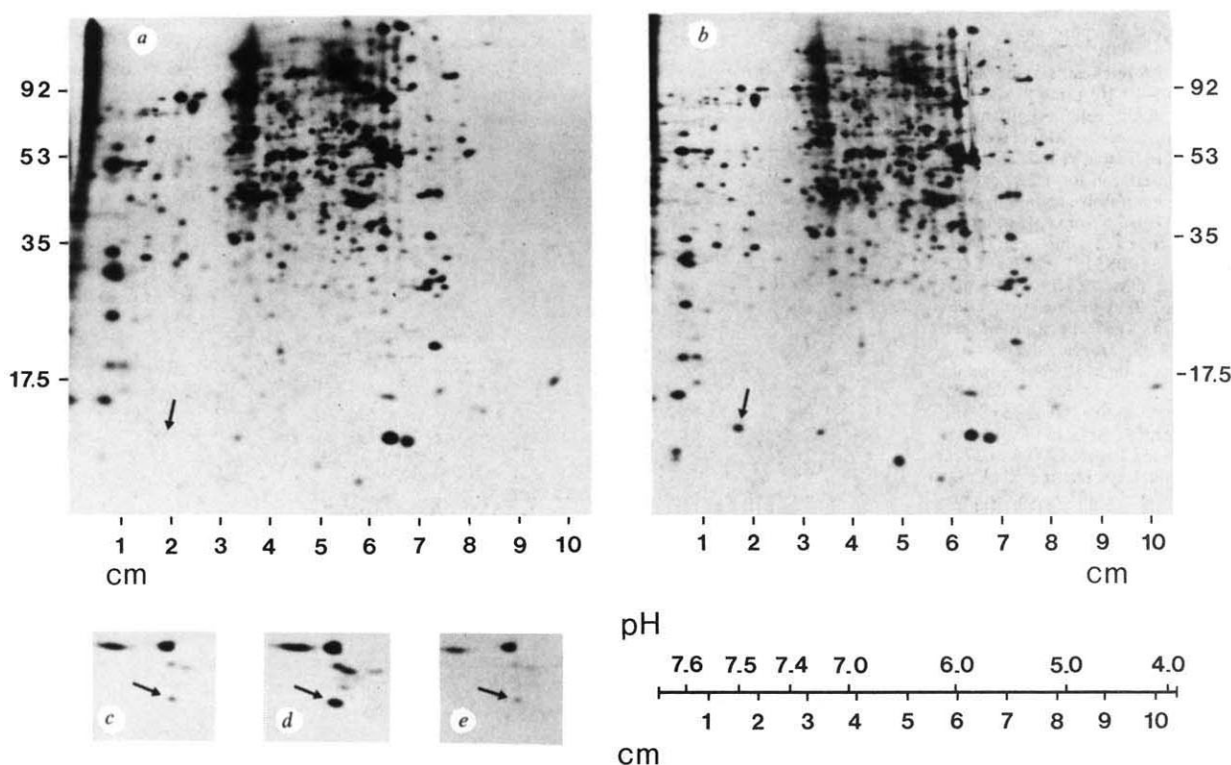


Fig. 3 EAT cells were treated with interferon and actinomycin D as below and then labelled with 60 µCi ml⁻¹ of a mixture of ³⁵S-methionine and cysteine for 1 h. At the end of the labelling the medium was removed, the monolayer was washed with ice-cold PBS and the cells were lysed in 0.4 ml of 9 M urea, 2% NP-40 per 60-mm dish. The viscous lysate was sonicated for about 15 s to disperse the DNA. Samples of 10 µl of the lysate were made up to 9 M urea, 2% ampholines (pH 3–10, Bio-Rad), 1% NP-40, 2.5% 2-mercaptoethanol and analysed by 2D gel electrophoresis as in Fig. 2 except that in *c*, *d* and *e* non-gradient 12.5% gels were used for the second dimension. Radioactive proteins were detected by fluorography. *a*, *c*, Control cells; *b*, *d*, cells treated with 500 U ml⁻¹ interferon for 7.5 h. *e*, cells treated with 500 U ml⁻¹ interferon and 2 µg ml⁻¹ actinomycin D for 7.5 h. The pH gradient in the isoelectric focusing gel was measured with a Bio-Rad propHiler and the equivalence of pH and the cm scale is shown. The shallow pH gradient at the basic end of the gel has been noted previously by O'Farrell¹⁷. Parts *a*, *b* were from the same experiment as Fig. 2, and *c*, *d* and *e* from another. In *c*, *d* and *e* only the region of the gel containing the induced protein is shown. The vertical scale in *a*, *b* shows the positions to which standard proteins (molecular weights in kilodaltons) migrated on the gel.

(500 U ml⁻¹) for 7.5 h and then incubated with ³⁵S-labelled amino acids for 1 h. The labelled proteins from the cells were analysed by 2D gel electrophoresis and the pattern of radioactive proteins was compared with that derived from similarly labelled control cells (Fig. 3a, b). The interferon-treated cells show strongly increased labelling of a protein which has the same mobility as the protein observed in Fig. 2 to be present in increased amounts in the translation products of the mRNA from interferon-treated cells. There are other proteins on the 2D gels whose labelling sometimes seems to be affected *in vivo* by interferon treatment but we have not fully established conditions for the changes in labelling of these proteins or found any changes in the corresponding mRNA levels. If cells are treated as above with interferon (Fig. 3d) except that actinomycin D is added at the same time as the interferon (Fig. 3e), the labelling of the 14,500-MW protein is the same as in the control cells (Fig. 3c). When cells are treated with interferon for various lengths of time then incubated with radioactive amino acids and analysed as above on 2D gels, the induction can just be observed after 5 h of interferon treatment and seems to be maximal by 9 h of interferon treatment (data not shown).

We scanned the spots on the fluorograms of the 2D gels and compared the relative blackening on the films to obtain an approximate estimate of the degree of induction of the 14,500-MW protein. This results in values of 7.5-fold for the labelled cells and five-fold for the *in vitro* translations.

We have not yet been able to equate this induced protein with any of the enzymes known to increase in activity after interferon treatment. We have not found any difference in double-stranded RNA-dependent protein kinase activity⁶⁻⁸ in the translation products of the mRNA from control and interferon-treated cells (data not shown). Nor do we detect any difference in the ability of the translation products to synthesise the 2,5A class of compounds⁹. The 14,500-MW protein does not appear to be mouse globin (determined by co-electrophoresis) or interferon (assayed by antiviral activity). As 500 U ml⁻¹ interferon slows the growth of these cells by less than 5% in the first 5 days of treatment, it seems unlikely that the induction we have observed is an indirect consequence of an inhibition of cell growth by interferon. We presume that our induced protein is different from a 56,000-MW protein recently reported by Ball¹⁰ to be induced in chick cells by interferon and we are currently trying to establish the function of the induced 14,500-MW protein and to examine other cell types for similar phenomena.

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Hydroxylamine stimulates carboxylase activity and inhibits oxygenase activity of cyanobacterial RuBP carboxylase/oxygenase

RIBULOSE-1,5-BISPHOSPHATE (RuBP) carboxylase/oxygenase (EC 4.1.1.39) catalyses both the photofixation of CO₂ and light-dependent O₂ uptake (photorespiration) in photo-trophs¹⁻⁴. Photorespiration is generally considered to be a wasteful process, reducing net plant productivity^{5,6} and N₂ fixation where it occurs⁷. The selective inhibition of RuBP oxygenase activity, either by endogenous metabolites or exogenous effectors, while allowing carboxylation to continue, has been viewed as a means of increasing plant productivity^{5,6}. There is no previous unequivocal evidence of one metabolite differentially affecting both reactions. Here, we present data on how it is possible, *in vitro*, to stimulate carboxylase activity and inhibit oxygenase activity simultaneously in extracts of the cyanobacterium *Anabaena cylindrica* by adding hydroxylamine, an intermediate in the reduction of nitrate to ammonia in cyanobacteria⁸ and higher plants⁹.

Anabaena cylindrica Lemm. (1403/2a) (Culture Centre of Algae and Protozoa, Cambridge, UK) was grown in pure culture as described previously¹⁰. RuBP carboxylase activity, measured as RuBP-dependent ¹⁴CO₂ incorporation into acid-stable material, and RuBP oxygenase activity, assayed as RuBP-dependent O₂ consumption, were followed simultaneously in an O₂ electrode. Preincubation for 10 min at between 0 and 40 °C had no effect on either RuBP carboxylase or oxygenase activities subsequently assayed at 30 °C. However, a 60% increase in both activities of the enzyme occurred after incubation at 50 °C for 10 min, before assay at 30 °C. The ratio of oxygenase/carboxylase activity remained fairly constant at around 0.063, irrespective of the temperature within the range 0-50 °C. This is the first evidence of heat activation of the enzyme from a cyanobacterium and accords with the finding of heat activation of the eukaryotic enzyme¹¹⁻¹⁴. Experiments detailed below were carried out after pretreatment of the *A. cylindrica* enzyme at 50 °C for 10 min, followed by equilibration at 30 °C for 2 min.

Table 1 Effects of various nitrogen compounds on the RuBP carboxylase/oxygenase activities of *Anabaena cylindrica*

Nitrogen compound added	RuBP carboxylase		RuBP oxygenase	
	1.25 mM*	10 mM*	1.25 mM*	10 mM*
None	100	100	100	100
KNO ₃	102	101	93	86
KNO ₂	82	88	72	79
NH ₂ OH	NT	171	NT	72
NH ₄ Cl	94	101	89	95

The preincubation temperature was 50 °C and the assays were carried out in the presence of 0.14 mM RuBP; other experimental details as in legend to Fig. 1. Activities are expressed as a percentage of those measured in the absence of nitrogen compounds.

* Final concentration of added nitrogen compound; NT, not tested.

Data on the effect of various nitrogen compounds on RuBP carboxylase/oxygenase activities (Table 1) show that of the compounds tested only hydroxylamine had any significant effect, stimulating carboxylase activity markedly and inhibiting oxygenase activity. Figure 1 shows the differential effects of hydroxylamine on *A. cylindrica* RuBP carboxylase and oxygenase activities at various effector concentrations. RuBP oxygenase was again inhibited by hydroxylamine, while carboxylase

was simultaneously enhanced when the hydroxylamine concentration was increased up to 10 mM. The differential effect of hydroxylamine on these reactions was greater in the presence of 0.14 mM RuBP than at 0.28 mM RuBP.

Our findings with hydroxylamine show similarities to and differences from the results of Bhagwat *et al.*¹⁵ using the spinach enzyme. Like them, we found an inhibition of RuBP oxygenase activity by hydroxylamine, but also, unlike them, we found a stimulation of RuBP carboxylase activity by hydroxylamine. Although this may reflect a difference between the prokaryotic and higher plant enzyme, it could also be due to the assay methods used. In particular, the Indian workers¹⁵ used separate

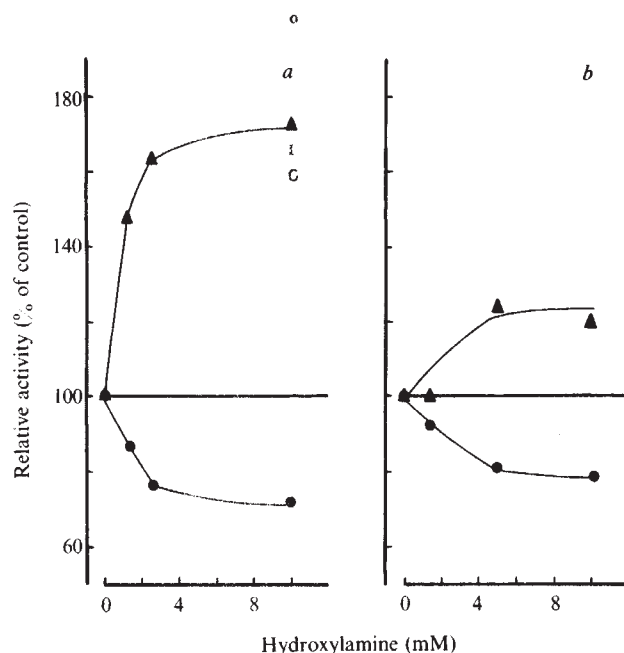


Fig. 1 Effects of increasing hydroxylamine concentrations on *Anabaena cylindrica* RuBP carboxylase/oxygenase activities at two RuBP concentrations. 8 l of log phase culture were collected by centrifugation, washed and resuspended in 2 vol. buffer (20 mM Tris, 1 mM Na₂EDTA, 10 mM MgCl₂, 50 mM NaHCO₃, 0.5 mM dithiothreitol and 1 mM phenylmethylsulphonyl fluoride, pH 8.0). Cells were disrupted by French pressure cell treatment at 110 MPa at 4 °C. The extract was then centrifuged at 15,000g for 20 min and the supernatant re-centrifuged at 100,000g for 60 min. After (NH₄)₂SO₄ precipitation of the supernatant, the 25–45% fraction was resuspended in buffer and concentrated by dialysis against buffer. This served as the enzyme preparation. Heat activation was carried out on 125-μl samples of enzyme in 1.5-ml capped vials to prevent loss of CO₂. The vials were incubated at 50 °C for 10 min, then maintained at 30 °C, the assay temperature, for 2 min before assay. RuBP carboxylase and oxygenase activities were measured simultaneously using a Pt-Ag oxygen electrode (Rank). Assays were started by adding 50 μl enzyme (10–40 μg protein) to the reaction mixture containing 100 mM Bicine-NaOH buffer, pH 8.3 (equilibrated with CO₂-free air), 0.27 mM NaH¹⁴CO₃, 20 mM MgCl₂ and 0.14 mM or 0.28 mM RuBP. In the final assay concentrations produced (21% O₂, 2.56 mM NaHCO₃ at pH 8.3) carboxylase and oxygenase activities were simultaneously followed. Oxygen uptake was linear for at least the first 100 s and data presented are those obtained over this 100-s period. Carboxylase activity was determined, after 100 s, by removing a 100-μl aliquot of the reaction mixture into trichloroacetic acid (final concentration 40% w/v). ¹⁴C incorporated into acid-stable material was then counted. Controls without RuBP were included. *a*, 0.14 mM RuBP; *b*, 0.28 mM RuBP; ▲, RuBP carboxylase; ●, RuBP oxygenase. Activities are expressed as a percentage of those measured minus hydroxylamine. Specific activities, measured minus hydroxylamine were: RuBP carboxylase, 8.13 and 12.13 μmol CO₂ fixed per mg protein per h at 0.14 and 0.28 mM RuBP respectively, and RuBP oxygenase, 0.88 and 0.90 μmol O₂ consumed per mg protein per h at 0.14 and 0.28 mM RuBP respectively.

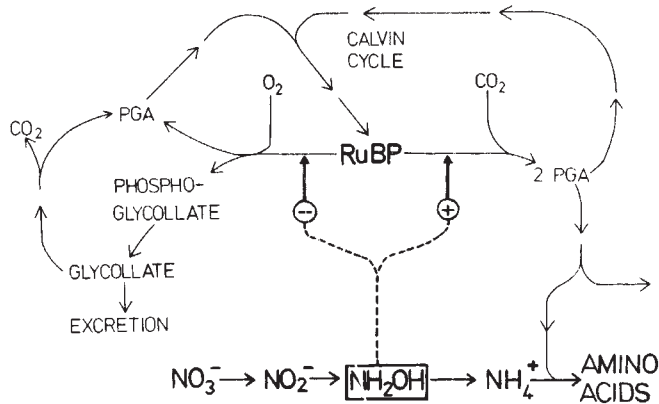


Fig. 2 Possible interactions between hydroxylamine and RuBP carboxylase/oxygenase based on our *in vitro* data. Solid lines represent metabolic pathways, broken lines represent inhibition (-) or activation (+) of enzymatic activities. PGA, 3-phosphoglyceric acid.

RuBP carboxylase and oxygenase assays and saturating bicarbonate and O₂ concentrations, starting both reactions by the addition of RuBP. We measured RuBP carboxylase and oxygenase activities simultaneously in the same vessel using assays with sub-optimal concentrations of CO₂ and O₂ respectively, and started the reactions by the addition of heat-activated enzyme. The effect of hydroxylamine on RuBP carboxylase may depend on CO₂ concentration, as well as on RuBP concentration. It has also been suggested that high CO₂ concentrations may protect the spinach RuBP carboxylase¹⁵ and our data support this contention.

The results point to a possible close coordination of nitrogen and carbon metabolism in *Anabaena*, as Solomonson and Spehar have postulated for *Chlorella*¹⁶. The latter workers suggest that hydroxylamine, produced during nitrate assimilation, combines with glyoxylate, produced by glycolate oxidation, to form glyoxylate oxime, and thence cyanide. Cyanide, produced in photorespiratory conditions, may thus effect the reversible inhibition of nitrate reductase at low concentrations. However, although cyanide production by *Chlorella vulgaris* has been demonstrated¹⁷ and cyanide inhibits *Chlorella variegata* nitrate reductase *in vitro*¹⁸, this inhibition does not occur when nitrate is present (see Table 4 of ref. 18). The inhibition of RuBP oxygenase by hydroxylamine would serve to reduce photorespiratory glyoxylate production and, according to the model of Solomonson and Spehar¹⁶, cyanide production. Our data, showing the direct inhibition of RuBP oxygenase by hydroxylamine, suggest that a regulation of photorespiratory carbon flow and nitrate assimilation may occur without the involvement of cyanide.

In vivo, the provision of adequate carbon skeletons is essential to prevent uncoupling by high pools of ammonia (Y. Steinitz, G.A.C. and W.D.P.S. in preparation). Our data suggest that in the presence of nitrate, hydroxylamine may contribute to the fine coordinate control of ammonia levels and the supply of carbon skeletons, as outlined in Fig. 2. The stimulatory effect of hydroxylamine on RuBP carboxylase and its concomitant inhibition of photorespiration would increase net carbon gain, thus helping to ensure the adequate supply of carbon skeletons for amino acid biosynthesis. The *in vivo* significance of these findings will depend on the size of the intracellular pools of hydroxylamine, or if hydroxylamine remains enzyme bound¹⁹, on an association between RuBP carboxylase/oxygenase and nitrate/nitrite reductase(s), or both. There is no evidence yet for a close association of such enzymes in cyanobacteria but there is evidence for such an association in eukaryotic systems^{20–22}.

It is also known that nitrate stimulates O₂ evolution when supplied to algae incubated in CO₂-limited conditions and this

has been attributed to nitrate acting as an alternative electron acceptor to CO₂ (ref. 23). Our data suggest that if hydroxylamine inhibits RuBP oxygenase activity *in vivo* this would decrease O₂ uptake and be responsible, in part at least, for the enhancement of O₂ evolution.

Irrespective of their *in vivo* significance, our data establish the fact that the RuBP carboxylase/oxygenase from an oxygenic photosynthetic prokaryote can be differentially regulated by chemical means.

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Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria

NITROGEN FIXATION by endosymbiotic cyanobacteria (blue-green algae) results in an important input of nitrogen into terrestrial ecosystems¹; however, there is as yet little information on its significance in the marine environment^{2,3}. Symbiosis of cyanobacteria with marine animals, although rare, is known to occur in an echiuroid worm⁴ and in sponges from coral reefs⁵ and the Mediterranean⁶. In the present study of sponge–cyanobacterial symbioses, several sponges from a coral reef in the Red Sea were tested, using the acetylene reduction technique⁷, immediately after their collection on reef-based platforms for their ability to fix nitrogen. Nitrogenase activity was detected in two sponges with cyanobacteria but not in a third with no cyanobacteria. This is the first demonstration of nitrogenase activity in an animal with symbiotic cyanobacteria. In two previous reports of nitrogen fixation in marine animals, the activity was attributed to bacteria in the gut^{8,9}. Although these preliminary experiments are not sufficient to assess the significance of cyanobacterial nitrogenase activity in sponges, it is possible that any additional fixed nitrogen would be beneficial

to sponges in tropical waters which are low in available nitrogen¹⁰ and in particulate nutrients^{11,12}.

Three sponges, two containing cyanobacteria and one without, were collected from illuminated habitats at a depth of 6–7 m on Harvey Reef adjacent to Port Sudan. The consistent presence of unicellular cyanobacteria, similar to those observed in other coral reef sponges⁵, was confirmed by electron microscopy. The cyanobacteria were observed, along with other bacteria, in large vacuolated cells, termed bacteriocytes¹³, throughout the tissue of *Siphonochalina tabernacula* (Row) (Fig. 1). The cyanobacteria were present in low numbers and are estimated to occupy less than 1% of the sponge volume seen in the electron micrographs. Similar cyanobacteria were observed in the thin brown pigmented ectosome of *Theonella swinhoei* Gray where they constitute 10–15% of the ectosome volume. These cyanobacteria are mostly free in the intercellular matrix with some present in specialised amoebocytes, termed cyanocytes⁵. Cyanobacteria numbers decrease away from the ectosome and are not evident in the deeper part of the endosome. Symbiotic bacteria are rare in the ectosome and particularly numerous in the endosome. Large populations of bacteria are the only symbionts observed in *Inodes erecta* (Keller).

Nitrogenase activity was determined using the acetylene reduction test as modified by Flett *et al.*¹⁴, except that the acetylene gas phase was maintained over the liquid phase because the sponge tissue was too fragile to agitate. Tissue samples were suspended in filtered seawater and incubated in 100-ml glass syringes at a depth of 30 cm in seawater exposed to saturating illumination (full sunlight, 5×10^{16} quanta cm⁻² s⁻¹) and at temperatures 1–4 °C above the ambient 31 °C. Duplicate 5-ml gas samples were collected in pre-evacuated tubes ('vacutainers') and estimated for ethylene content by gas chromatography at laboratories in the University of Bristol and Westfield College, London. After correcting for initial contamination, the ethylene concentration was determined using a variation of Henry's law¹⁴.

When similarly sized pieces of *S. tabernacula* and *I. erecta*, collected from similar habitats, were incubated with acetylene, ethylene was produced in *S. tabernacula* and apparently consumed in *I. erecta* (Fig. 2a). Adjacent pieces from a large *S. tabernacula* specimen were incubated in light or dark, with considerably more ethylene being produced in the light sample (Fig. 2b). The rate of production of ethylene in *S. tabernacula* was linear during the initial 2 h of incubation, with rates slightly decreasing in the subsequent period (Fig. 2c). In similarly sized tissue pieces of *S. tabernacula* incubated in the light with DCMU (dichlorophenyl dimethyl-urea), a specific inhibitor of photosystem II reaction having no effect on animal tissue, no appreciable reduction in total ethylene production was observed during the incubation period (Fig. 2c). The presence of DCMU had an apparently stimulatory effect on the O₂-sensitive nitrogenase by depressing photosynthetic O₂ evolution, although this effect was probably compensated for later by the shortage of photosynthetically produced reductant¹⁵. In both dark and DCMU treatments, nitrogenase activity in the cyanobacteria may have persisted at the expense of preformed or non-photosynthetic source of reductant¹⁶, or activity could have been partly or fully bacterial in origin. In *T. swinhoei*, ethylene production in tissue samples containing between 20% and 30% pigmented ectosome was higher than in the non-pigmented endosome (Fig. 2d).

Nitrogenase activity is thought to be associated with the symbiotic cyanobacteria rather than other bacteria, for the following reasons: (1) nitrogenase activity was evident in *S. tabernacula* and *T. swinhoei*, which harbour symbiotic cyanobacteria, and was absent in *I. erecta*, which lacks cyanobacteria; (2) more ethylene was produced by the ectosome of *T. swinhoei*, which contained cyanobacteria, than by the endosome almost free of cyanobacteria; (3) nitrogenase activity was higher in illuminated tissue than in tissue incubated in the dark; and (4) the amounts of ethylene produced in *S. tabernacula* and

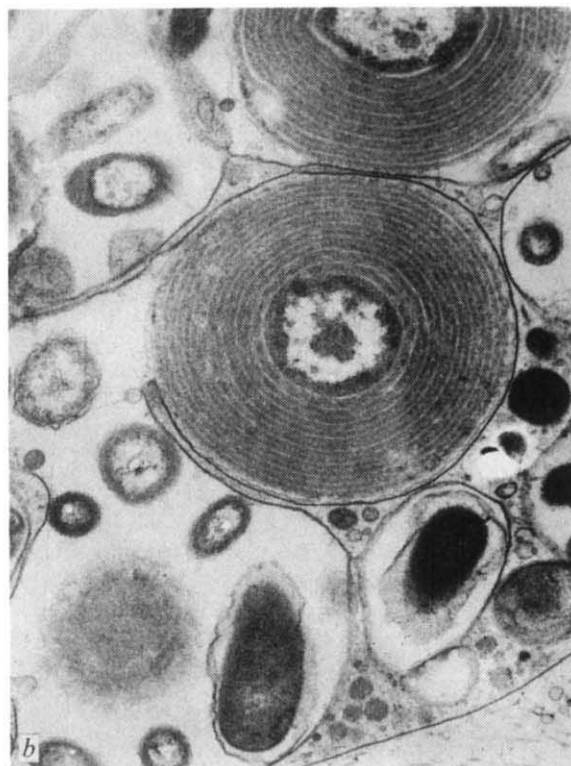


Fig. 1 *a*, Electron micrograph showing cyanobacteria (C) and bacteria (B) within sponge cell vacuoles in the intercellular matrix of *Siphonochalina tabernacula*. Tissue was fixed in 2.5% glutaraldehyde in seawater:water (4:1), post-fixed in 1% OsO₄ and embedded in Spurr's resin. $\times 30,000$. *b*, Section through sponge vacuole showing cyanobacterium with spiral thylakoid and adjacent smaller bacteria. $\times 15,000$.

T. swinhoei were comparable although the bacterial population in *T. swinhoei* was many times larger than that in *S. tabernacula* and similar to that in *I. erecta*.

It is unlikely that nitrogenase activity was due to exogenous microorganisms colonising the surface, as the sponge epithelial cells (exo- and endopinacocytes) readily phagocytose particulate matter adhering to the surface¹².

Although we conclude that the nitrogenase activity measured was due mainly or solely to symbiotic cyanobacteria, the role of other bacterial symbionts must be considered. Mixed populations of bacteria occur in sponges^{5,13}, including phototrophic anaerobes¹⁷, and it is possible that some of these bacteria display nitrogenase activity. Associations between aquatic cyanobacteria and heterotrophic bacteria were considered to be beneficial to both partners¹⁸. Bacteria possibly enhance nitrogen fixation in freshwater cyanobacteria by creating a reducing atmosphere¹⁹. Synergistic nitrogen fixation may occur in sponges when cyanobacteria and heterotrophic bacteria are closely associated (Fig. 1).

More detailed experiments are required to establish the nutritional and ecological significance of the nitrogenase activity determined in these preliminary experiments. Although at first sight the levels of activity, 2–4 nmol C₂H₄ produced per g (wet weight of sponge) per h for *S. tabernacula* and 3 nmol C₂H₄ per g per h for *T. swinhoei*, seem to be rather low, it should be remembered that they originate from small populations of cyanobacteria in a tissue containing large quantities of intercellular matrix and inert skeleton. Comparisons with data obtained with other systems are not possible until the cyanobacterial population may be reliably estimated, and the cyanobacteria isolated and tested in pure culture.

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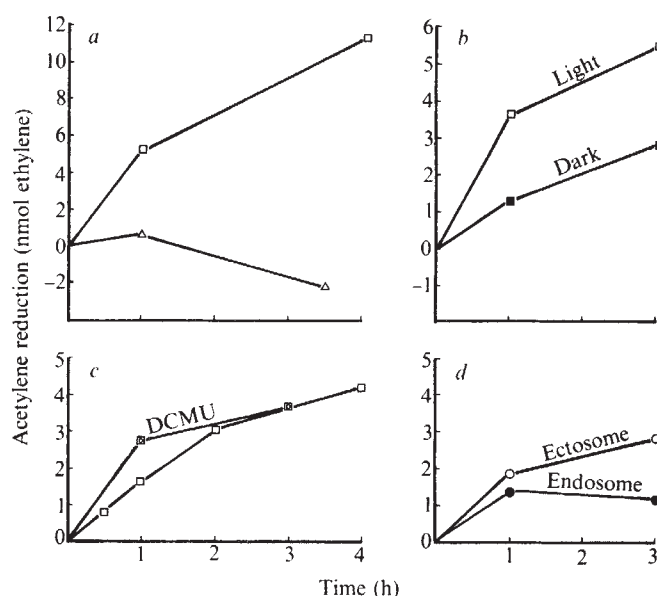


Fig. 2 Acetylene reduction (nmol of ethylene in 5-ml gas samples): *a*, by equal (8.1 g) tissue samples of *Siphonochalina tabernacula* and *Inodes erecta* (no cyanobacteria) incubated in light; *b* during light and dark incubation of equal (3.4 g) samples of *S. tabernacula*; *c*, by equal (3.9 g) pieces of *S. tabernacula* incubated in the light in the presence or absence of 10⁻⁵ M DCMU; *d*, by 2.1 g of tissue containing pigmented ectosome and 1.7 g of white endosome of *Theonella swinhoei*. Squares, *S. tabernacula*; triangles, *I. erecta*; circles, *T. swinhoei*.

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Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell

SOME viruses are known to persist despite a functional antiviral immune response by the host. For example, measles or measles-like viruses can cause persistent infection in man leading to a progressive degenerative disease, subacute sclerosing panencephalitis (SSPE), characterised by unusually high titres of measles virus antibodies in serum and cerebral spinal fluids (see refs 1, 2 for reviews). Peripheral blood lymphocytes specifically responsive to measles virus antigens are generated and lyse target cells expressing virus antigens on their surfaces^{3,4}. Sera from SSPE patients enhance rather than block or suppress immunologically mediated killing^{3,5}. But although this anti-measles virus immune response is vigorous, the virus infection does not terminate because one can detect virus antigens and isolate infectious virus from central nervous system and lymphoid tissues¹. To explain this we postulated that antibody to measles virus modulates or strips viral antigens off surfaces of virus infected cells^{6–7}. This contention is based on the finding that specific measles antibody added to cultured infected cells modulate viral antigens on the cells' surfaces⁶. These cells then express less viral antigens on their surfaces and thereby avoid the host's immune assault. Cells denuded of viral antigens on their surfaces resist antibody and complement-mediated or immune lymphocyte-mediated killing^{6,7} yet retain viral genetic information⁶. Further, during antibody modulation *in vitro*, the numbers of viral nucleocapsids accumulated inside the cell dramatically increase and are positioned randomly⁶. This *in vitro* picture closely resembles the distribution of nucleocapsids in cells obtained by biopsy from patients with SSPE^{1,8,9}. Quantitatively, about 10- to 50-fold less antibody is needed on the surfaces of infected cells to modulate viral antigens than is needed to activate the complement system leading to immune lysis of infected cells^{3,10}. To clarify the molecular events occurring during antibody-induced antigenic modulation, we have

studied measles virus antibody bound to the surfaces of infected cells and ascertained what alterations occur within these cells. Here we report that measles virus antibodies added to virus infected HeLa cells decrease the content of both viral structural polypeptide haemolysin expressed on the cell surface and a structural polypeptide (measles virus phosphoprotein, P) found inside the cell. These observations indicate that antibody directed against an antigen on the cell surface can interfere with viral proteins expressed inside the cell. Because phosphoprotein seems to form complexes with the nucleocapsid, our findings may offer leads towards understanding viral regulation during persistence. We also note that three measles virus polypeptides—P, nucleocapsid (NC) and matrix protein (M)—are phosphorylated.

HeLa cells acutely infected with measles virus were cultured with and without antibody to measles virus in the media (see Fig. 1 for experimental details). The antibody used for modulation reacted with all measles virus structural proteins as determined by immune precipitation and analysis on polyacrylamide gel electrophoresis (PAGE). The antibody neutralised 4.5×10^5 PFU per ml. After being cultured in antibody for 6 or 18 h⁶, HeLa cells were collected, washed four times in serum-free medium, and incubated in media free of either methionine or phosphorus. After 1 h the cells were washed and incubated in methionine- or phosphorus-free media to which ³⁵S-methionine or ³²P was added. Cells were collected 2 h later from both the controls (infected-unmodulated: NMOD) and the infected modulated (MOD) cultures, washed and lysed with 2% NP40 in TNE buffer. Nuclei and cell debris were removed by centrifugation and the cytosol preparations were incubated with

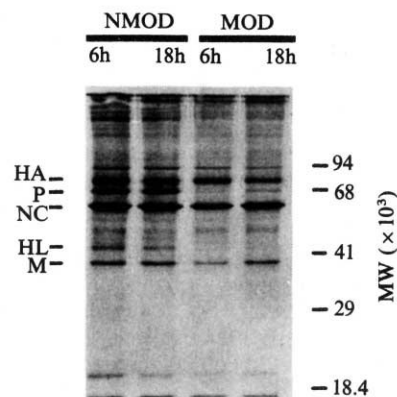


Fig. 1 HeLa cells were infected in suspension with Edmonston strain of measles virus at a multiplicity of infection of 1. Cells were pelleted 24 h later and resuspended in Eagle's minimal essential media (MEM) containing either fetal calf serum (FCS) (non-modulated, NMOD) or antibodies to measles virus (modulated, MOD) for 1 h. The cells were pelleted and suspended in fresh MEM with 20% serum containing antibodies to measles virus or 20% FCS and placed on a rotating platform at 37°C. At 6 h and 18 h, cells were collected by low-speed centrifugation, washed four times with serum-free MEM and incubated in methionine-free media. After 1 h in which $10 \mu\text{Ci } ^{35}\text{S}$ -methionine per ml was added and the cells incubated at 37°C, the cells were washed once with phosphate-buffered saline and lysed at 4°C with NP40 in TNE buffer containing 1 mM PMSF. Nuclei and cell debris were removed 15 min later by centrifugation at 1,000g for 15 min, and the cytosols (300 μl) were precipitated with 10 μl hyperimmune antibody to measles virus. Incubation lasted overnight at 4°C. Thereafter, an excess of heat-inactivated, formalin-fixed *Staph. aureus* was added to precipitate all the formed complexes. Precipitates were washed twice in 0.5% NP40 in TNE buffer and twice again with 0.02% NP40 in buffer, suspended in sample preparation buffer containing 1% SDS and 1% 2-mercaptoethanol and immersed in boiling water for 2 min. The bacteria were pelleted at 1,000g for 15 min and supernatants removed. The immune precipitates were counted and applied to 10.5% SDS gels for PAGE analysis. Gels were stained, fixed and dried on filter paper. Kodak XRP-1 X-ray film was exposed to the dried gels and developed. Appropriate molecular weight markers were run in adjacent lanes. Data shown are representative of seven such experiments.

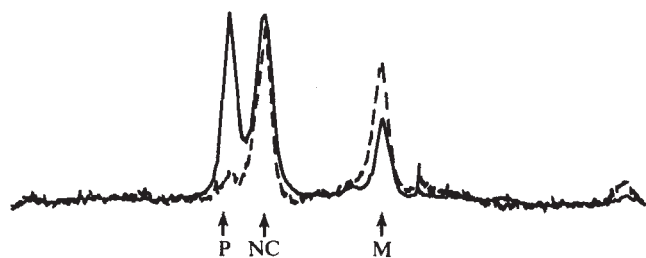


Fig. 2 HeLa cells infected with measles virus were treated as described in Fig. 1 except that phosphorous-free media and ^{32}P ($50 \mu\text{Ci ml}^{-1}$) replaced the ^{35}S -methionine and cells were collected 12 h post-infection. The ordinate represent relative density and the abscissa the relative migration. The three phosphorylated measles virus polypeptides seen are: phosphoprotein (P); nucleocapsid (NC) and matrix protein (M). Solid line, ^{32}P non-modulated; broken line, ^{32}P modulated.

antibodies to measles virus overnight at 4°C . The antibody used for immune precipitation recognised all structural measles virus polypeptides found in the virion and in infected cells. The antigen-antibody complexes formed were then precipitated with *Staphylococcus aureus*, washed, dissolved in 1% SDS and 1% 2-mercaptoethanol and electrophoresed on SDS-PAGE in conditions designed to segregate measles virus polypeptides. In the conditions used, structural measles virus polypeptides were immunoprecipitated and co-migrated with purified measles virion polypeptides. Measles virus polypeptides were identified by tryptic peptide mapping¹¹. Experimental controls showed that (1) measles virus antibody did not precipitate proteins in uninfected cells that migrated in positions of measles virus polypeptides and (2) sera free of antibodies to measles virus did not precipitate measles virus polypeptides found in infected cells.

Figure 1 shows the five structural polypeptides of measles virus demonstrated when HeLa cells were incubated only in fetal calf serum (NMOD) but not antibody. These are the haemagglutinin (HA), the phosphoprotein (P), the nucleocapsid (NC), the haemolysin (HL) and the matrix protein (M). Only the HA and HL were expressed on the cells' surfaces as detected by lactoperoxidase ^{125}I -labelling of living cells¹² and previous observations¹³. In contrast, HeLa cells incubated with antibody to measles virus synthesised equivalent amounts of three of the five proteins, namely HA, NC and M as studied with ^{35}S -methionine label (Fig. 1). Hence, in the experimental conditions used, modulation for 18 h or less, detectable amounts of HL, a surface protein, were markedly diminished by the presence of anti-measles antibody, whereas the other surface protein, HA, was unchanged. In addition, cytosol preparations from antibody modulated infected cells contained less P protein than unmodulated cultures. These results were reproducible and quantitated by both densitometer scans and counting the radioactivity contained within the labelled viral polypeptide. HL decreased by 70% or more and P by 80% or more in the preparations from modulated cells compared to those that were not modulated by antibody. Quantities of NC in modulated and unmodulated cells varied less than 10%. We performed a series of experiments in which the conditions of electrophoresis were altered (7–15% gels with and without urea), several human and rabbit sera containing measles antibody were used to modulate or precipitate, and purified IgG from these human and rabbit sera were used. All gave results similar to those shown in Fig. 1. Failure to decrease the amount of HA expressed on the surfaces of infected cells was unexpected, and probably due to the brief time (<18 h) for which infected cells were modulated. Previous experiments⁷ showed that after 48 h of antibody-induced modulation, viral antigens were no longer detected on the cells' surface by either radiolabelled binding or immune cytotoxicity assays. When IgG from sera without antibody to measles virus was added to the cultures in identical conditions, neither HL or P protein were altered in concentration.

We next determined whether antibody(s) to non-viral determinants expressed on the surface of virus-infected cells also decreased the HL and/or P protein of measles virus. For these experiments, measles virus-infected HeLa cells were incubated with antibodies directed to the HeLa cell surface in conditions like those used for modulation. Although antibodies to HeLa cell surface components bound to antigens expressed on the membranes of virus-infected HeLa cells for up to 18 h in culture, the quantitative expression of the five viral polypeptides remained unaltered compared to cultures in which HeLa antibodies were absent or fetal calf serum was substituted. HL reacted correspondingly. Hence, the decreases in P and HL directly reflected the fact that antibodies to measles virus bound to measles virus determinants expressed on the cell surface.

P protein is a phosphorylated protein¹⁴ and we next studied the incorporation of ^{32}P into this protein during antibody modulation. Figure 2 shows a densitometer scan of immune precipitates obtained from cells labelled with ^{32}P and autoradiographed. In unmodulated cells, P, NC and M polypeptides all incorporated ^{32}P , indicating that these three measles virus structural proteins are phosphorylated. Comparatively, in modulated cells, only trace amounts of ^{32}P were incorporated into P polypeptides while an enhancement of ^{32}P was incorporated by the M protein. No difference in incorporation of ^{32}P was noted for NCs studied in modulated and unmodulated cells. Labelling studies with ^{32}P and ^{35}S studies were run concurrently (Figs 1 and 2). Changes in the phosphorylation of M protein might alter NC recognition and alignment at the plasma membrane, thereby inhibiting viral maturation. Such alterations in phosphorylation could change the migrational characteristics of M protein on PAGE and may explain, in part, the differences in migration of M protein reported by others with SSPE material^{15,16}.

Thus our results show that specific antibody can bind to the surface of an infected cell and not only strip off surface viral determinants but also alter viral polypeptide(s) found inside the cell not associated with the plasma membrane. It is not clear whether there is a decrease in synthesis, increase in degradation, or alteration of the P protein structure that would render it non-antigenic or change its mRNA. Nevertheless, it is clear that antibodies to a specific virus determinant on the cell surface can send a signal transmembrally that alters cytoplasmic viral events. Furthermore, the P protein, or its analogue in other viral systems, seems to be associated with the transcriptase complex^{17,18}. The sequence of events described here could lead to alterations of measles viral synthesis leading to persistent infection.

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***In utero* sister chromatid exchange analysis for detection of transplacental mutagens**

FETAL exposure to agents which damage DNA can result in birth anomalies, cancer and inherited abnormalities. In the present report we describe a new approach for the detection of fetal DNA damage through the enumeration of sister chromatid exchanges (SCE) in mouse fetal chromosomes. SCE can be identified as reciprocal exchanges of fluorescent intensities between sister chromatids in metaphase cells which have divided twice in the presence of bromodeoxyuride (BrdU) (Fig. 1). Analysis of SCE has been shown to be a sensitive and reproducible means of detecting chemical mutagens and carcinogens¹⁻⁹.

Differential chromatid staining of metaphase cells from maternal and fetal tissues was achieved when pregnant females were intravenously infused with BrdU for 24 h at gestational days 11, 15 and 19. SCE frequencies were simultaneously studied in fetal cells as well as in maternal bone marrow. Baseline SCE frequencies in fetal cells were generally lower than in maternal cells, ranging from 2.4 to 3.6 SCE per second replication cycle cell, and these values did not change significantly as a function of gestational age (Table 1, Fig. 1b).

To examine *in utero* SCE induction, three known mutagens (cyclophosphamide, CP; mitomycin C, MMC; adriamycin, ADM) were injected into pregnant females on day 13 of gestation (Table 1, Fig. 1a). Drug concentrations were selected which would induce similar levels of SCE for each agent in maternal cells (approximately 20 SCE per cell¹⁰). These same drug doses resulted in varying levels of SCE induction in fetal cells. Cyclophosphamide administration resulted in similar SCE induction in fetal and maternal cells; MMC resulted in fetal SCE levels which were slightly lower than those in maternal cells, while fetal SCE induction with ADM was only one third that seen in maternal cells (but significantly higher than baseline levels).

The doses of CP and MMC which resulted in a 5-10-fold increase in fetal SCE were well below the teratogenic doses for these drugs^{11,12} while ADM, which showed the weakest induction of fetal SCE, is not teratogenic¹³. We therefore suggest that *in utero* SCE analysis should be used in addition to existing assays for screening fetal exposure to mutagens, carcinogens and teratogens which act at the level of DNA damage. *In utero* SCE analysis is relatively simple, rapid,

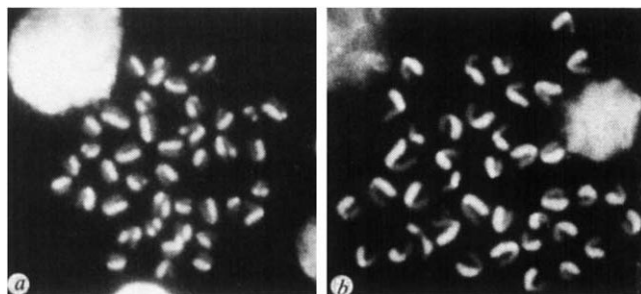


Fig. 1 Photomicrographs of mouse fetal chromosomes. *b*, Second replication cycle cell showing baseline level of SCE; *a*, second replication cycle cell showing cyclophosphamide induced level of SCE. Two to four month-old female C57BL/6J mice (Jackson) were mated with male mice of the same age and strain. Gestational age was determined by counting the number of days after observation of vaginal plugs or by examination of fetuses at the time of killing. Pregnant mice were infused according to our previously described techniques at 50 mg per kg wt per h (refs 8, 15). After 23 h of intravenous infusion, mice were injected with 2.5 µg demecolcine (Colcemid, GIBCO) and killed 1 h later. Those mice treated with mutagens (CP, Mead, Johnson; MMC, Sigma; ADM, Adria Labs) were injected intravenously 1 h after the onset of the infusion. After killing, the entire uterus was excised and individual fetuses incubated in 0.2% collagenase (GIBCO). Vigorous pipetting in Eagle's minimal essential medium (MEM) resulted in single cell suspensions of fetal tissues. Cells were isolated from maternal bone marrow as described previously^{8,15}. Cell suspensions were then swollen in 0.07 M KCl, fixed (methanol/acetic acid, 3:1) and dropped on to glass slides. Chromosome preparations were stained with 4'-6-diamidino-2-phenylindole (10 µg ml⁻¹, ref. 16) and chromosomal analyses were performed with a Zeiss photomicroscope equipped with epi-illumination.

reproducible, requires small numbers of animals, allows simultaneous examination of the effect of agents on fetal and maternal cells, and may be more sensitive than previously described techniques such as the micronucleus assay¹⁴ or measurement of chromosomal aberrations¹.

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Table 1 Baseline and mutagen-induced SCE frequencies in maternal and fetal cells

Gestational day*	Drug† (mg per kg)	Bone marrow‡	Embryos§
11	0	4.0 ± 0.4	2.7 ± 0.2 (4)
15	0	3.2 ± 0.4	2.4 ± 0.3 (4)
19	0	4.8 ± 0.5	3.6 ± 0.6 (3)
13	CP 10	23.4 ± 1.1	27.0 ± 1.4 (4)
14-15	CP 10	22.0 ± 1.4	25.0 ± 1.2 (4)
13	MMC 1	18.2 ± 0.9	14.2 ± 1.1 (3)
13	MMC 1	21.2 ± 1.0	15.8 ± 1.0 (3)
13	ADM 5	17.8 ± 1.2	6.3 ± 0.4 (4)
13	ADM 5	17.6 ± 0.9	6.5 ± 0.4 (4)

* In cases where a range of days are indicated, gestational age was estimated by examination of fetuses at the time of killing.

† Drugs were intravenously injected in a volume of 0.1 ml.

‡ A minimum of 25 second replication cycle bone marrow cells were analysed for each mother.

§ A minimum of 10 second replication cycle cells were analysed for each fetus. Values in brackets indicate the number of fetuses analysed.

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The pair of central tubules rotates during ciliary beat in *Paramecium*

UNLIKE the one-dimensional movement of striated muscle, the beat of a cilium is typically three dimensional¹. Thus, the dynein arms, which are situated on the peripheral tubules around the circumference as well as longitudinally along the cilium, must be temporally coordinated in their actions. The mechanism for coordination is not known. The present study was undertaken to see whether the pair of central microtubules exhibits any systematic movement during the ciliary beat. We conclude that the central pair of tubules rotates anticlockwise 360° per beat cycle and that this rotation may regulate the dynein arms. *Paramecium* cilia were used because: markers distinguish the two central tubules so that their orientation can be unambiguously determined; and the metachronal waves of cilia can be 'instantaneously fixed' for the analysis of sequential phases of the beat.

The force required for motion of cilia, eukaryotic flagella and sperm tails is generated by the dynein arms, which cause sliding of adjacent peripheral microtubule doublets²⁻⁴. The dynein arms have been shown to contain Mg²⁺-ATPases⁵. The force generated by the arms can only cause the adjacent doublets to move tipward⁶. As the dynein arms are arranged in a ninefold radial symmetry, a force generated on one side will be cancelled by that on the opposite side. Thus, the physical arrangement necessitates a regulatory mechanism that allows some of the dynein arms to work while others do not. The central pair of the 9 + 2 microtubular structure is a candidate for at least part of this regulatory mechanism for several reasons. First, orientation of the central pair is correlated with beat direction. The central pair is orientated at right angles to the power stroke in a wide variety of metazoan cilia^{7,8}. Gibbons⁸ noted that in the Protozoa *Paramecium* and *Opalina*, the central-pair orientation was not uniform. Tamm and Horridge⁹ then showed that the orientation of the central pair was perpendicular to the direction of bending, even when the effective stroke direction was changed. Second, various *Chlamydomonas* mutants missing one or both of the central tubules or even some accessory components of the central-tubule complex are found to be paralysed¹⁰. In these mutants, the dynein function, as assayed by peripheral microtubule sliding, remains normal¹¹. Third, studies have indicated some interactions between the radial spoke of the peripheral doublets and the projections from the central pair. It has been suggested that such interactions convert sliding to bending¹². When the radial spokes are deleted by mutations, the cilia are also paralysed^{11,26}.

As in *Tetrahymena*¹³, the two central tubules in *Paramecium* can be distinguished by a 20-nm spur on one tubule (Fig. 1a). The two central tubules also terminate at different levels near the ciliary base (Fig. 1b), as shown by Dute and Kung¹⁴. Using both of these morphological markers, we have examined the orientations of central pairs near the bases of cilia on serial sections of the *Paramecium* surface. We have examined 119 central pairs in which both the insertion into the axosome and the 20-nm spur could be scored. A vast majority of these, 108 central pairs, show that the tubule which is inserted into the axosome also bears the spur (Fig. 1a, b). Of the remaining 11 cases, eight are ambiguous because different tubules were scored to have the spur in different serial sections. Only three cases show that the tubule with the spur is not inserted into the axosome. This observation makes the recent speculation by Hausmann and Fischer-Defoy²¹ that there is alternate sliding of central tubules unlikely, and also increases our confidence in tubule identification. Using the spur and/or the axosomal insertion as markers, a vector can be drawn from the tubule without a marker to the tubule with the marker(s). The angular changes of this vector can then be studied.

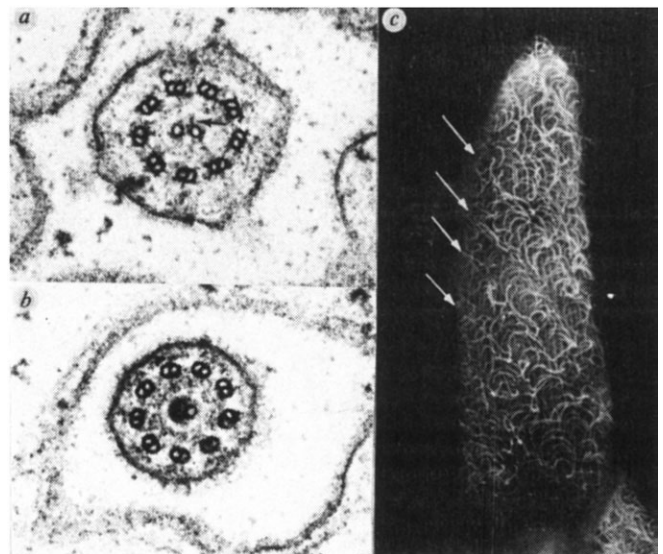


Fig. 1 a, Cross-section of a *Paramecium* cilium showing the 20-nm spur (arrow), $\times 44,850$. b, The same cilium as in a in more proximal section showing the tubule with the spur entering the axosome. The axosome is seen as an electron dense material around the tubule in cross-section, $\times 44,850$. c, Scanning electron micrograph of an instantaneously fixed *Paramecium* showing metachronal waves (arrows), $\times 552$. Wild-type *Paramecium tetraurelia*, 51s, were grown in Cerophyl medium bacterised with *Enterobacter aerogenes*¹⁵. The cells were washed then fixed while swimming in 4 mM CaCl₂, 1 mM Tris-HCl pH 7.2. An excess of instantaneous fixation solution (S. L. Tamm, personal communication) with final concentration of 2% OsO₄, 2% glutaraldehyde, 0.05 M Na-K phosphate pH 7.2 was pipetted on to cells and left at room temperature for 10–15 min. The cells were washed, post-fixed in 0.5% uranyl acetate and dehydrated through ethanol series. For scanning electron microscopy the cells were critical-point dried in 20- μ m mesh Nitex bags, put on stubs, coated with carbon-platinum and viewed on a JSM U3 scanning electron microscope. For transmission electron microscopy, cells were embedded in Epon-araldite¹⁶ or in Spurr's¹⁷ and sectioned on a Reichert OmU3 ultramicrotome. The sections were stained either with 0.5% uranyl magnesium acetate then lead citrate¹⁸ or 1% potassium permanganate¹⁹ then lead citrate²⁰. Serial sections were photographed on a Phillips 300 electron microscope then enlarged photographically before examination.

Each beat cycle of the *Paramecium* cilia has a planar power stroke and a three-dimensional recovery stroke²². The cilia of an actively swimming *Paramecium* do not all beat in synchrony. Adjacent cilia in certain directions are slightly out of phase so that metachronal waves (Fig. 1c) are propagated. These waves give us an opportunity to study ciliary beat. Cilia at the same phase of beat form a line of synchrony parallel to the direction of the power stroke. Perpendicular to this line of synchrony, and in the direction of wave propagation, cilia are in progressively earlier phases of the beat (Fig. 2). Cilia just before the power stroke are at the wave crests (position 1, Fig. 2). The wave troughs (position 2) are made by cilia at the beginning of their recovery stroke which comprises most of the wave (positions 3–5). In *Paramecia* that have been 'instantaneously fixed', the waves are well preserved with cilia frozen in various phases of their beat (Fig. 1c). Our strategy has been to reconstruct the events during the ciliary beat cycle by examining the ultrastructure and orientation of central pairs of cilia along the line of wave propagation.

To examine the possible angular changes of the vector of the central pair, micrographs of the 20 to 30 serial sections near a *Paramecium* surface were traced, with cross-sections of all cilia outlined. The tubules with the spur and/or axosomal insertion were marked and the vectors drawn. Each cilium was numbered on each of the serial sections and the numbering was maintained through the series. An arbitrary line constant for all sections was

used to measure the angles of the vectors. The measured angles were grouped into nine 40° sectors. The classification of the angles into nine sectors is arbitrary but reflects the impracticability of measuring the angles with greater accuracy. The angle and coordinates for each cilium from all the serial sections were compiled into one map. The angles measured from all the cilia in the sections are not constant but span all the nine possible sectors. The best-fit set of parallel lines through groups of cilia with the same sector angle was drawn using a computerised co-variance analysis. In the more distal sections of the series, cilia could be seen in various cross, glancing or longitudinal views, depending on the phases of their beats. A survey of the pattern of these images of cilia enabled us to estimate the lines of synchrony. We found that the lines of synchrony estimated by this method parallel the lines of best-fit drawn from the angular data.

The orientation of each ciliary central pair near the base is plotted against its distance from a fixed computer-drawn line of synchrony (Fig. 3). Neighbouring cilia with central pairs in the same orientation are grouped (brackets in Fig. 3). In one typical case, shown in Fig. 3, the mean distance between neighbouring central pairs within groups is only $1.4 \pm 0.7 \mu\text{m}$ (mean $\pm$ s.d.; range $0-3.5 \mu\text{m}$), while the mean distance between groups of the same orientation is $6.7 \pm 1.2 \mu\text{m}$ (range $6-12 \mu\text{m}$).

The central-pair orientation of cilia located along the perpendicular of the line of synchrony changes systematically in the anticlockwise direction (looking from tip to base) as one proceeds in the sequence of their beat cycle. This shift in orientation is continuous, with the exception of a few of the cilia in the cell in Fig. 3. The curvature of the *Paramecium* surface, the inaccuracy in aligning serial sections, the inability to score and include the vector for all the cilia and the imperfection of the metachronal waves themselves, all contribute to departure from the ideal.

We can also estimate the approximate wavelengths using the more distal sections described above, where cilia are seen in various views depending on the phases of their beat. In the case shown in Fig. 3, the wavelength is between 7 and $12 \mu\text{m}$. This is similar to the distance of $6.7 \pm 1.2 \mu\text{m}$ (range $6-12 \mu\text{m}$) between cilia having the same central-tubule orientation. Similar results have been obtained in four other *Paramecia*.

In the opposite direction from wave propagation, cilia are frozen at phases of the temporal sequence of their beat (Fig. 2)²². For a *Paramecium* fixed during forward swimming, this direction is from its anterior left to posterior right. Along this same direction we see that the central pairs rotate anticlockwise.

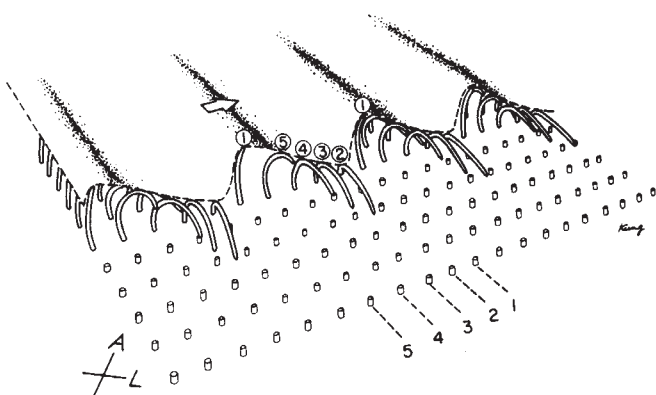


Fig. 2 Stylised diagram of metachronal waves of cilia on the surface of *Paramecium*. Phases of ciliary beat are numbered 1-5. The power stroke is from 1 to 2 and the recovery stroke from 2, 3, 4, 5 back to 1. For a forward-swimming *Paramecium*, the power stroke is from anterior right to posterior left (refer to compass; A, anterior; L, left). This is also the direction of the lines of synchrony (dotted lines), along which cilia are at the same phase of their beat. Perpendicular to these lines of synchrony is the direction of wave propagation (broad arrow) which is opposite the direction of cilia at subsequent phases of their beat.

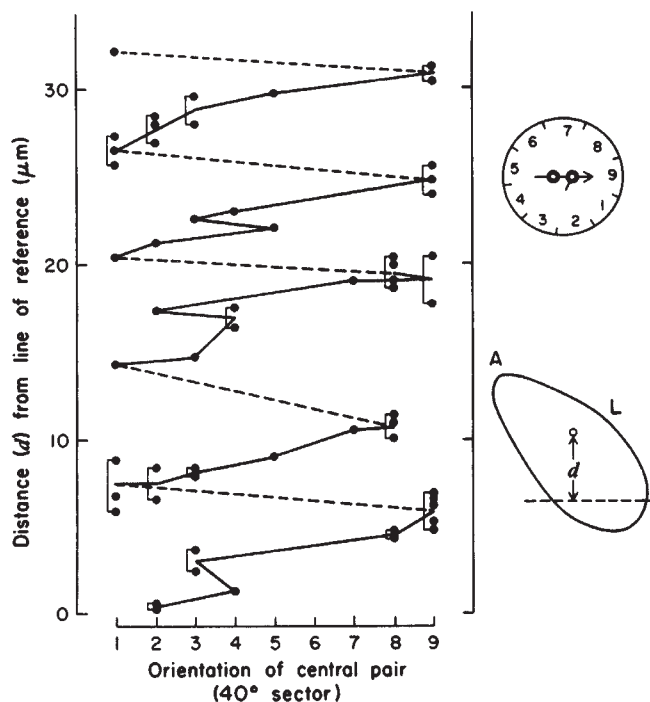


Fig. 3 Orientations of the central pairs plotted against the distances of the cilia from a line of reference on the surface of *Paramecium* fixed during forward movement. The line of reference (dotted line of lower inset) parallels the lines of synchrony along which cilia are in the same phase of their beat (see text and Fig. 2). In forward swimming *Paramecium* this line is from its anterior (A) right to posterior left (L). The distances (d) are measured in μm . The orientation of the central pair is represented by a vector drawn from the tubule without a spur to the one with (upper inset). The orientation is classified into nine categories as the vector falls into various 40° sectors. The sectors are numbered clockwise (looking from tip to base) and arranged such that the vector parallels the line of reference at sector 9. Neighbouring cilia with the same central-pair orientation within half a wavelength independently estimated from more distal sections (see text) are grouped (vertical brackets). The line is drawn through cilia or groups of cilia of successively increasing distance (d) from the reference line. Note that, in general, the central-pair orientation is successively more clockwise as d increases. Five complete revolutions are seen. As d increases in the direction of wave propagation we encounter cilia frozen in successively earlier phases of the ciliary beat in this direction (see text and Fig. 2), and we conclude that the central pair rotates anticlockwise during the beat.

Thus, one simple explanation is that each central pair rotates anticlockwise during the beat. Furthermore, as the wavelength (estimated from distal sections) and the distance between groups of central tubules having the same orientation are similar, one may conclude that there is a 360° rotation with each beat cycle.

As shown in Fig. 3, there are far more central pairs orientated at sectors 8, 9, 1, 2 than at sectors 4, 5, 6, 7. Such an uneven distribution may be related to the temporal asymmetry of the ciliary beat cycle, that is, far more time is spent in the recovery stroke than in the effective stroke. We are now in the process of correlating the orientations of the central pairs to specific phases of the beat cycle.

An alternative interpretation of the data is that the central pair rotates in one direction for several sectors and then quickly rotates back, instead of continuously rotating in one direction. This would explain why few cilia orientate at certain sectors. However, the possibility is unlikely, as, without exception, the central-pair orientation of 27 individual cilia has been found to be progressively more anticlockwise when we examine the progressively more proximal sections of the same cilia. We have also examined $0.25-0.5 \mu\text{m}$ -thick sections of *Paramecium* cilia under a high-voltage electron microscope and have found twists

in the central pairs. If the rotation of the central pair is generated at the proximal end, a twist may form and propagate distally along the cilium when the pair encounters some resistance along its length.

Regardless of whether the rotation is continuous or discontinuous, these results refer only to the proximal region of the central pairs. As we do not have markers on the peripheral tubules, we cannot determine their movement. Rotation of the whole axonemal assembly within which the central pair is fixed is not likely. Preliminary evidence from thick section high-voltage electron micrographs indicates that the assembly of the nine peripheral tubules also shows twists. However, the twist of the peripheral tubule assembly does not correlate with that of the central pair. Indeed, stereomicrographs of thick sections show that the twist of the central pair is opposite that of the peripheral tubule assembly in some portions of the cilia.

The 360° rotation of the central pair might be unique to a certain class of cilia or, on the other hand, it might be a phenomenon common to many types of cilia with the 9+2 structure. Variation in the orientation of the central pair has been reported. Satir²³ noted differences of more than 90° in the orientation of the central pair in active cilia from *Elliptio* gill fixed instantaneously. In *Opalina* Tamm and Horridge⁹ reported that the orientation of the central pair is always at right angles to the direction of bending and that this orientation shifts by almost 90° at the trailing edge of the metachronal wave crests. As neither study used cilia with distinguishable central tubules, the angle variation observed is a minimal estimate.

We propose a model in which the central pair regulates the sliding of peripheral tubules. In this model, the rotation of the central pair acts as a distributor to signal peripheral tubule activity in sequence. This signal may be transmitted to the peripheral doublets through the radial spokes. The rotation of the central pair may generate the twist and cause its helical propagation towards the tip. This model would predict that the period of bend propagation is identical to the period of rotation. Observations with high-speed cinematography by Jarosch and Fuchs²⁴ of the flagellar motion in *Synura* are consistent with this prediction. This model may also provide the physical basis for the helical propagation of force-generating sites along the cilium postulated by Hiramoto and Baba²⁵. The model can be tested by first showing a rotation of the central pair independent of peripheral tubule sliding. This may be done by using cilia whose dynein has been chemically inhibited or genetically removed. The model can be further tested by correlating the central-pair orientation with local dynein ATPase activation or cross-bridge formation.

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Increased incidence of abnormal nasal cilia in patients with retinitis pigmentosa

RETINITIS PIGMENTOSA (RP) is the name given to a group of inherited eye disorders of unknown aetiology, whose symptoms are loss of night vision and constriction of the field of vision. The conditions are progressive and eventually in some cases blindness occurs¹. Although there are certain syndromes which include RP, for example, the Laurence-Moon-Biedl¹, or Cockayne's syndrome², in most cases the disease is considered to be localised to the eye. However, a high incidence of deafness has always been associated with RP^{1,4} and Massof and Finklestein (personal communication) have found that at least 15% of their cases have inner ear deafness not associated with presbycusis or acoustic trauma. As the outer limbs of photoreceptors are modified cilia³ and the sensory epithelium of the inner ear is derived from ciliated epithelium, we wondered whether RP might be associated with a more general defect of ciliated structures. Accordingly we examined samples of nasal mucosa from 11 patients with RP and report here an increased incidence of cilia with abnormal axonemal microtubular structures, and also of compound cilia. Although our patient sample was small, it was heterogeneous and our findings may therefore apply to patients of different genetic type. If, as seems plausible, the ciliary abnormalities are related to the disease process in the eye, nasal mucosa would offer an accessible source of human material for the study of basic pathological processes in photoreceptors.

All our patients had been investigated at the Retinal, Genetic, and Electrodiagnostic Clinics of Moorfields Eye Hospital. Six of the cases had been called 'Usher's syndrome' (autosomal recessive RP with deafness⁴) although in some cases the deafness was not profound, or was of very early onset. In two cases, the

Table 1 Relative numbers of abnormal axonemal patterns in patients and normal controls

Controls (N = 11)			RP patients (N = 11)		
9+2 & 9+0	'Tips'	Others (abnormal)	9+2 & 9+0	'Tips'	Others (abnormal)
254	6	13*	219	3	59*

The absence of the central two singlet microtubules (9+0) implies that the section was made near the base of the cilium, and is not an indication of abnormality. Likewise, sections through the tips of the cilia ('Tips', counted in separate column) show single microtubules symmetrically arranged about part or all of the circumference of the cilium. In the controls, between 0 and 19% of abnormalities were seen in various individuals: in the patients, the range was between 6 and 37%. See Table 2 for statistics.

* $\chi^2 = 31.77$, highly significant.

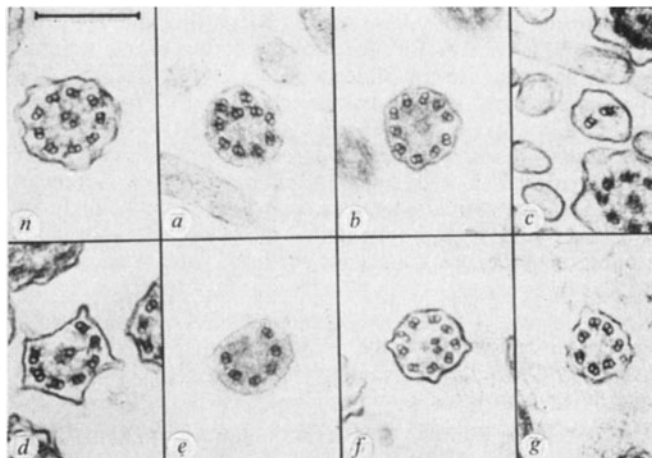


Fig. 1 Transmission electron micrographs of normal and abnormal axonemes of cilia in transverse section. *n*, Normal 9+2 arrangement. *a*–*g*, Abnormal arrangements of microtubules, all from RP patients. Scale bar, 0.25 μ m.

inheritance was autosomal dominant: these patients did not complain of deafness and audiometry has not been carried out. In three patients, the RP seemed to be 'sporadic' and associated with deafness. In one of these deafness was inherited dominantly but the proband was the only member of the family with RP. On this basis, and considering the nature of the disease in each patient, we classify the cases as eight recessive and three dominant. The 11 controls were reasonably well matched for sex and age. Samples of mucosa were taken from the inferior turbinate of the nose under either local anaesthesia (in RP patients and four of the controls) or general anaesthesia (the remainder of the controls). The tissue was immediately pinned flat on a piece of cork, with the ciliated surface uppermost, and placed in Karnovsky fixative to which 2 mmol of MgSO_4 had been added to improve the visibility of the dynein arms⁵. After overnight fixation, the tissue was washed in buffer and postfixed in osmium tetroxide, followed by block staining with uranyl acetate. Tissue was dehydrated, mounted in 'Araldite' and sectioned. Sections were stained with uranyl acetate and lead citrate, and examined in a Phillips 201 electron microscope.

Electron micrographs of transverse sections of cilia were taken at a final magnification of 65,000–180,000. Only those cilia in which a microtubular pattern was clearly seen were counted. In some cases in which there were few precisely transverse sections of cilia, the gonioscopic stage was used to ensure that circular outlines were obtained.

In normal human material there are few departures from the classic 9+2 arrangement of the axonemal microtubules (Fig. 1, *n*). The abnormalities we saw in the patients' material consist of both increases (Fig. 1, *a* and *b*), decreases (Fig. 1, *c*–*g*) and irregular arrangements of the doublets (Fig. 1, *a* and *b*). There may also be displacement of the two central singlet microtubules (Fig. 1*f*). In one outline (not shown) we found five single microtubules centrally in the axoneme. Using the most conservative criteria, we find >20% of cilia from our patients to be abnormal, compared to 3% from the controls (Table 1).

Table 2 Percentage of abnormal axonemal structures

<i>Normal subjects</i>			
<i>N</i>	$\bar{X}$	s.e.m.	
11	3.55%	0.84%	
	(Subgroup with no nasal abnormality)		
5	3.21%	2.5%	
<i>RP patients</i>			
11	22.84%	3.84%	
	(Subgroup with dominant inheritance)		
3	23.73%	4.8%	

Table 3 Compound cilia

	Control group	RP patients
No. of persons with no nasal abnormality	5	7
<i>a</i> Total length of section examined	607 μ m	734 μ m
<i>b</i> Length of ciliated cell		
mean	6.6	7.3
range	4.4–7.7	5.0–10.1
<i>c</i> Cilia/cell		
mean	70	89
range	36–125	23–113
<i>d</i> Estimated no. of cilia in sample	6,396	12,068
<i>e</i> No. of compound cilia in sample	0	39
<i>f</i> Proportion of compound cilia		
$\bar{X}$	0	0.86%
s.e.m.	—	0.32%
<i>g</i> Expected no. of compound cilia in control group	21 (from simple average), 55 (from value of $\bar{X}$) 13–96 ($\pm$ 2 s.e.m.'s)	

For each patient, several micrographs ($\times 10,800$) of ciliated epithelium were examined, of length shown in *a*. The length of that single cell associated with the largest number of transverse sections of cilia *b* was then measured. We counted the number of these cilia *c*. From *a* and *b* we calculate the number of cells in the sample, and hence the number of cilia in the sample *d*. This method overestimates the number of cilia. The total number of compound cilia associated with the entire sample is given in *e*. For each patient we were able to determine the proportion of compound cilia, *f*, and thus obtain the s.e.m., which should be normally distributed. Hence we can make predictions *g* about the number of compound cilia we expect to see in the control group, on the assumption that the differences between the two columns are solely due to sampling. The predictions and the experimental result are so different that we can reject this hypothesis.

Both dominant and recessive cases showed the abnormality (Table 2). One patient with autosomal recessive disease had only 6% abnormal axonemes (and other abnormalities, see below). We do not yet know whether this is a sampling error, or whether recessive RP patients fall into more than one group. Though the mean values given in Table 2 show that normal subjects and patients fall into separate groups, we cannot tell from this preliminary survey what the population ranges may be.

Large structures containing many sets of microtubules were seen in many of our patients. Although the incidence of such compound cilia was low ($\sim 0.3\%$) the appearances were bizarre and striking (Fig. 2).

Compound cilia occur in the olfactory system of some fish⁶ and in patients with upper respiratory tract infections and allergies⁷ (B.F., in preparation). We have therefore excluded from our sample any control or RP patient who had evidence of such conditions. This reduced the number of the control group to five, three of whose tissue was obtained during the course of operation for deviated nasal septum, and two healthy volunteers. Four of the RP patients were excluded on the same grounds. No compound cilia were seen in the normal material, but 39 were counted in the sections made from the RP patients' mucosa, in five of the seven cases. The Fisher exact probability that this result could occur by chance is very low, $P = 0.026$. However, there are very few compound cilia, even in our patients, and we have therefore attempted more detailed morphometry, as described in Table 3, which shows that the patients have normal sized ciliated cells, and that the total number of cilia is not reduced. Although the incidence of compound cilia is small, given the length of ciliated epithelium examined, there is only a very small probability that the patients and normal material could be drawn from the same population. In one of the two patients in whom we saw no compound cilia, a large sample was obtained, and, using the data from Table 3, we calculate that we should have seen 10 compound cilia, if the incidence was the same in this patient as in the remainder. It is

thus possible that RP patients do not form a uniform population so far as the incidence of compound cilia is concerned.

Apart from deafness, 10 of our cases of RP have difficulties with balance. In one further case (not reported here) we have found abnormalities of the cilia of the uterine tube. Moreover, in Cockayne's syndrome, there is an unexplained predisposition to respiratory infection which could be due to a ciliary defect². The defects of cilia in our patients may be generalised and the loss of photoreceptors could be due to the same basic cause. However, there is evidence that the pigment epithelium, and not the rods, is defective in some animal retinal dystrophies⁸ and perhaps in some patients^{9,10}. In this connection it seems possible that RP patients do not form a homogeneous group, and one or other or both of the defects shown in Figs 1 and 2 may be absent. This would be important in efforts to subdivide the group of diseases called retinitis pigmentosa, and to assess the number and frequency of abnormal genes^{11,12}. Our results do not show immediately how the abnormality detected could produce RP. The growth of rod disks, or the provision of metabolites for the rod outer segments may be associated with the disposition of microtubules in the narrow ciliary neck. The biochemistry of cilia and outer limbs is strikingly similar in many ways¹³⁻¹⁷, and thus cilia may form a useful model for the inaccessible photoreceptors. Research into RP has been handicapped by the lack of normal human ocular tissue, and the almost complete absence of suitable human pathological material¹⁸. In contrast, it should be possible to obtain large quantities of normal and abnormal human cilia fairly easily.

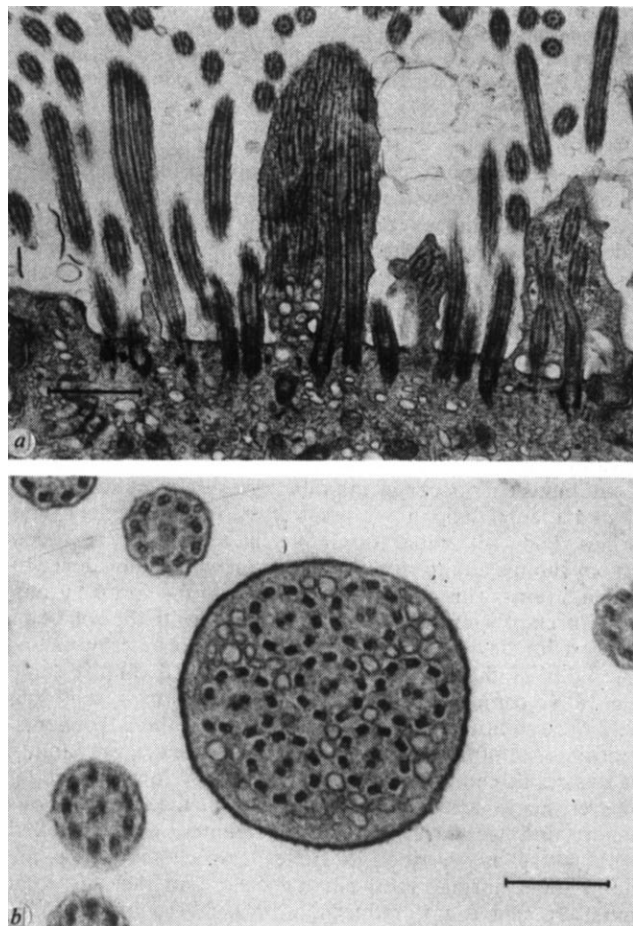


Fig. 2 *a*, Transmission electron micrograph showing longitudinal sections through compound cilia, from a patient with dominantly inherited RP. Scale bar, 1 μ m. *b*, Transmission electron micrograph of compound cilium in transverse section. Note the 7 microtubular patterns with a 9+2 arrangement. The single axoneme at '11 o'clock' has an additional peripheral microtubule. (RP patient, autosomal recessive inheritance.) Scale bar, 0.25 μ m.

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Note added in proof: We have recently investigated three patients with X-linked disease. All have ~40% abnormal axonemes, but two are females, in whom retinal functional defects are mild^{11,12,20}, implying that the degree of abnormality of photoreceptors and cilia is not necessarily the same.

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Membrane fluidity of a fatty acid auxotroph grown with palmitic acid

HOMEOVISCOUS adaptation, whereby the fatty acid composition and unsaturation of complex lipid bilayers in membranes may be adjusted to maintain fluidity irrespective of growth temperature, has been demonstrated for *Escherichia coli*¹, *Acholeplasma laidlawii*² and *Bacillus stearothermophilus*³. Even at high temperatures, a minimum amount of unsaturated fatty acid is required for growth of an *E. coli* auxotroph⁴, and generally, for *A. laidlawii* B, at least half the lipids must be in the fluid or disordered state to allow normal growth⁵. Unlike the fatty acid auxotrophs of *E. coli* and *A. laidlawii* used in previous investigations of membrane fluidity^{2,4,5}, a fatty acid auxotrophic *Butyrivibrio* sp. (strain S2) isolated from the ovine rumen was found to be unable to synthesise long-chain fatty acids and incorporated palmitic acid, without desaturation, into lipids of the plasmalogen type partially as a new long-chain dicarboxylic acid⁶. This gave an opportunity to study the lipid composition of a microorganism with a defined fatty acid availability, and also to study the state of fluidity of its membranes in the complete absence of acyl chain unsaturation. We report here a study of membrane lipid fluidity in *Butyrivibrio* S2 using the electron spin resonance (ESR) probe 5-doxylstearic acid. Considerable rigidity of hydrocarbon chains is demonstrated but a phase change or structural reorganisation occurs at the lowest temperature, allowing optimal growth of the organism.

After incorporation of [¹⁴C]palmitic acid into *Butyrivibrio* S2 the long-chain ¹⁴C-labelled hydrophobic moieties produced by methanolysis of the complex bacterial lipids (Table 1) consisted of: (1) about 7% cetyl alcohol; (2) some 40% of the dimethyl

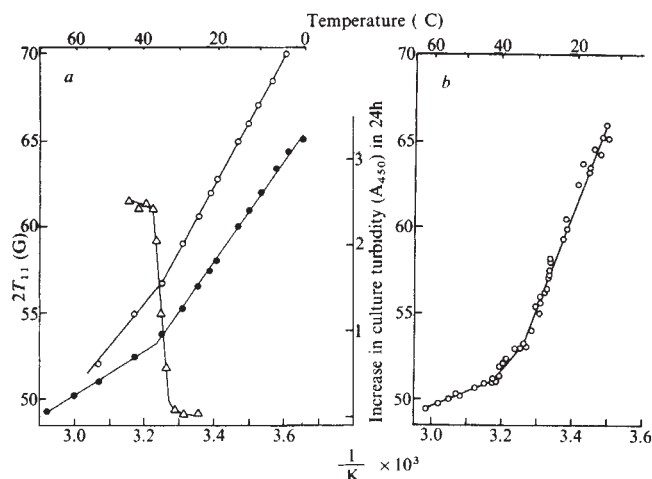


Fig. 1 *a*, Temperature dependence of maximum hyperfine splitting ($2T_{11}$) for 5-doxylstearic acid incorporated into whole cells (○) or extracted lipids (●) of *Butyrivibrio* S2 and the relationship between growth temperature and the increase in culture turbidity (Δ) in 24 h. *b*, Temperature dependence of maximum hyperfine splitting ($2T_{11}$) for 5-doxylstearic acid incorporated into multilamellar structures of dipalmitoyllecithin. The gel straight line is a least squares fit to the experimental data. The discontinuities at 33.5 and 41 °C are consistent with the pre- and main transition temperatures reported previously¹⁴. 5-Doxylstearic acid was mixed with the extracted bacterial lipids or dipalmitoyllecithin in chloroform/methanol (2:1 v/v) in the molar ratio of ~1:300. The mixed lipid was readily dispersed in D₂O by a method described previously¹⁵. In spin-label experiments using whole cells, residual cysteine from the growth medium which could destroy the nitroxide radical^{3,16}, was removed by washing the cells four times with 0.1 M phosphate buffer, pH 7.4. The 5-doxylstearic acid (0.5–1% of the calculated dry weight of lipid in the cells, assuming an average molecular weight of 1,500) was dried down from chloroform/methanol (2:1 v/v) solution in a round-bottom flask and the dry film was incubated with the cell suspension with shaking for 15 min. ESR measurements were carried out with a Varian E 104A (X band) spectrometer equipped with a variable temperature accessory ($\pm 1^\circ$ heating rate 20°C h^{-1}). Samples were contained in 0.1 ml glass or quartz capillaries accommodated within standard 4 mm quartz ESR tubes. Bacterial growth was determined turbidimetrically (A_{450}).

ester of a new type of long-chain dicarboxylic acid which had been formed in the bacterium by a dehydrogenation condensation reaction involving two palmitic acid molecules, and has recently been identified as 15,16-dimethyltriacontan-1,30-dioic acid (diabolic acid); (3) 41–42% of long-chain aldehyde (as the dimethylacetal derived from plasmalogen), consisting predominately of palmitaldehyde but with a little stearaldehyde; and (4) a smaller long-chain fatty acid methyl ester component (9–11%) consisting largely of palmitic acid but with a trace of stearic acid. The complex lipids also contained appreciable quantities of esterified but unlabelled butyric acid.

As with only palmitic acid available for growth, no unsaturation or cyclopropane moieties occur in the hydrophobic chains of *Butyrivibrio* S2, the bacterium possesses no fatty acid desaturase system. Furthermore, as the organism cannot synthesise long-chain fatty acid⁶, it is likely that the low concentrations of stearic acid and stearaldehyde present are derived from trace impurities of stearic acid in the palmitic acid or other medium components. No radioactivity was detected in the stearic acid when the organism was grown in the presence of [$1\text{-}^{14}\text{C}$]palmitic acid, indicating that it had not been formed by chain elongation.

The fluidity of the organism's membranes and the lipids extracted from them was examined by ESR using the probe 5-doxylstearic acid (*N*-oxyl-4',4'-dimethyloxazolidine derivative of 5-ketostearic acid, I(12, 3); Synvar). At all temperatures examined the spectra of the spin label present in either whole cells or the lipid dispersion were typical for highly anisotropic

motion. Table 2 shows a comparison of the published values of the maximum hyperfine splitting $2T_{11}$ for 5-doxylstearic acid bound to various cell membranes at temperatures between 15 and 25 °C; it is clear that *Butyrivibrio* S2 grown with a palmitic acid supplement shows a degree of order in its membrane matched only by the rigid, specialised purple membrane of *Halobacterium* spp. At the growth temperature of the organism (39 °C) the maximum hyperfine splitting was $2T_{11} = 55.5$ G (Fig. 1a), a value similar to that observed with the same label in dipalmitoyllecithin bilayers below the T_c (in the gel phase) (Fig. 1b), in which the hydrocarbon chains are packed in an orderly crystalline lattice.

The temperature dependence of the maximum hyperfine splitting ($2T_{11}$) of the spectrum of 5-doxylstearic acid bound to whole cells was compared with that obtained when the same label was incorporated into the lipids extracted from such cells. Plots of $2T_{11}$ against the reciprocal of absolute temperature (Fig. 1a) showed discontinuities at 34.5 °C for the whole cells and 35.5 °C for the extracted lipids, indicating some type of phase change or structural reorganisation in membrane lipids. This temperature coincided with the lowest temperature at which optimal growth occurred in a palmitate-supplemented medium (Fig. 1a). The single break in the Arrhenius plot (Fig. 1a) probably reflects the relative simplicity of the lipid hydrocarbon chains, which exhibit little variation in chain length, and no unsaturation or cyclopropane substitution as is usually seen in bacterial lipids. Clearly this latter heterogeneity in the fatty acid chains is unnecessary for bacterial growth, a conclusion also recently reached by Silvius and McElhaney⁸, using *A. laidlawii* B grown in the presence of antilipogenic agents.

It is significant that the break in the Arrhenius plot (Fig. 1a) coincides with the minimum temperature that allows optimal growth in a palmitate-supplemented medium. What are the structural changes that occur in the lipid bilayer at that temperature and are apparently necessary for membrane viability and vigorous growth of *Butyrivibrio* S2? The membrane lipids of *Butyrivibrio* S2 possess no unsaturation except the vinyl ether unsaturation in the plasmalogen chains. However, there is little evidence that plasmalogen when substituted for acyl chains can increase the fluidity of lipid bilayers^{9,10}.

Table 1 Long-chain hydrophobic moieties present in *Butyrivibrio* S2 grown with a palmitic acid supplement

Hydrophobic moieties produced	Structure	% Present	
		Expt 1	Expt 2
Alcohol	C16:0	6.77	6.00
Diabolic acid			
dimethyl ester	(C16:0) ₂	41.03	37.99
Dimethylacetal	C16:0	40.15	41.46
	C18:0	2.65	2.15
Fatty acid			
methyl ester	C16:0	8.91	11.06
	C18:0	0.48	1.33

Butyrivibrio S2, an obligate anaerobe, was cultured in a virtually fatty acid-free liquid medium containing 0.4% (w/v) galactose, as described previously⁶ (medium 3). The palmitic acid ($30\text{ }\mu\text{g ml}^{-1}$, and in some experiments [$1\text{-}^{14}\text{C}$]labelled) required to promote growth was dispersed in this medium with the aid of sodium taurocholate ($300\text{ }\mu\text{g ml}^{-1}$) except where extracted lipids or washed cells were to be used for ESR experiments, when a growth-limiting concentration of fatty acid ($20\text{ }\mu\text{g ml}^{-1}$) was dispersed in the medium by ultrasonication. Cultures were inoculated with 5% (by vol) inoculum, grown to maximum turbidity in the same medium, and were incubated at 39 °C for 16–18 h. Bacterial cells were collected by centrifugation (20,000g; 20 min) and lipids were extracted and analysed⁶. The complex lipids were hydrolysed with 5% (w/v) HCl in methanol (90 °C; 2 h) which converted any esterified long-chain carboxylic acids into their methyl esters and long-chain aldehydes (plasmalogens) into dimethylacetals while any alcohols were liberated as such. Quantification of the hydrophobic moieties was carried out by radioactivity measurements after TLC⁶ and the chain lengths present were determined by GLC.

Table 2 Maximum hyperfine splitting $2T_{11}$ of 5-doxylstearic acid spin label bound to various cell membranes

Membrane system	Temperature (°C)	$2T_{11}$ (G)	Reference
Human erythrocytes	23	55–60	17, 18
Human lymphocytes	23	53.7	17
L cells	23	54.7	17
Sarcoplasmic reticulum vesicles	22	53.8	19
<i>E. coli</i> membrane vesicles	20	56.7	20
Brush border membrane vesicles	23	60	Hauser, H., unpublished
Beef heart mitochondria	23	55.6	
<i>Mycoplasma laidlawii</i> membranes	15	58.0 (cells grown at 15 °C)	21
	15	62.5 (cells grown at 37 °C)	
<i>Halobacterium halobium</i> (purple membrane)	15	63.7	22
	20	63.0	
	25	62.0	
<i>Butyrivibrio</i> S2	15	65.0	Present work
	20	63.0	
	25	61.0	

There are two possible ways in which some limited degree of hydrocarbon chain motion could occur in the lipid bilayer. First, it has been shown here and previously¹¹ that many of the complex glycolipids and phospholipids produced by members of the genus *Butyrivibrio* contain at least one esterified butyryl group per molecule. Such short fatty acid chains are likely to increase the fluidity of a lipid bilayer. Second, the long-chain dicarboxylic acid (diabolic acid) occurring as an integral esterified part of the membrane's phospholipid contains two vicinal methyl groups substituted in the centre of the methylene chain⁷. Such methyl groups are again likely to perturb the orderly packing of the hydrocarbon chain in much the same way that anteiso methyl branched fatty acid molecules pack less readily and consequently have a much lower capillary melting point than their straight-chain or isobranched homologues^{12,13}. Indeed, the melting point of 41 °C observed⁷ for the dimethyl ester of the diabolic acid



isolated from palmitic acid grown *Butyrivibrio* S2 cells is much less than that predicted from the melting points of the homologous series of straight-chain dicarboxylic acid diesters. To a certain extent the role of the diabolic acid could be equivalent to that believed to be played by the high levels of anteiso branched fatty acyl groups found in psychrophilic microorganisms when compared with their closely related mesophilic counterparts¹². These acids, together with the greater percentage of unsaturated fatty acyl chains, help to maintain membrane fluidity at the lower growth temperature of the psychrophilic organisms. However, this fluidising effect of the vicinal methyl groups in diabolic acid might be partly counterbalanced by the very long hydrocarbon chain which may span the membrane bilayer thus reducing the cooperative lipid motion.

We believe that the present naturally-occurring auxotroph has survived in the rumen through its ability to make use of the abundance of saturated fatty acid (both palmitic and stearic acid) and *trans* octadecenoic acid formed by biohydrogenation occurring in this environment. When grown on palmitic acid, the bacterium incorporates this fatty acid exclusively into the lipid part of its membranes, resulting in a very rigid membrane

structure as indicated by the large hyperfine splitting (Table 2). Although *Butyrivibrio* S2 has no capacity to shorten chains or desaturate the fatty acids available from the environment or convert these into a cyclopropane form, it may nevertheless decrease the rigidity of its lipid bilayers by: (1) introducing butyryl groups produced by the fermentation of hexoses, and (2) introducing anteiso-type branching by a novel type of condensation reaction between two palmitic acid chains. These structural features are probably responsible for some kind of molecular motion in the lipid bilayer, which, though rather restricted, is sufficient to maintain the functions of the membrane at growth temperatures above 34 °C. The ESR measurements indicate that the membranes of the organism have a tightly packed, highly ordered lipid bilayer even at the physiological growth temperature of 39 °C. This suggests that in *Butyrivibrio* S2 a more limited degree of hydrocarbon chain motion is required for membrane viability than has been found in other bacteria.

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Cholesterol modulates activity of calcium-dependent ATPase of the sarcoplasmic reticulum

BIOMEMBRANES consist of an asymmetric lipid bilayer matrix into which and around which the various proteins are situated. The proteins may be attached to the outside of the lipid bilayer (extrinsic proteins), but in many cases the proteins (intrinsic proteins) are embedded within, and can span, the bilayer. Associated with this is the idea that in many cases the lipid matrix is in a fluid condition in which the lipids are essentially above their transition temperature (T_c) and able to diffuse along the bilayer length. The perturbation introduced into the lipid bilayer by the presence of an intrinsic protein has recently been discussed^{2,3}. Some workers^{4,5} have suggested that intrinsic proteins, for example the Ca^{2+} -ATPase of the sarcoplasmic reticulum, carry with them, even when excess bulk fluid lipid occurs, a

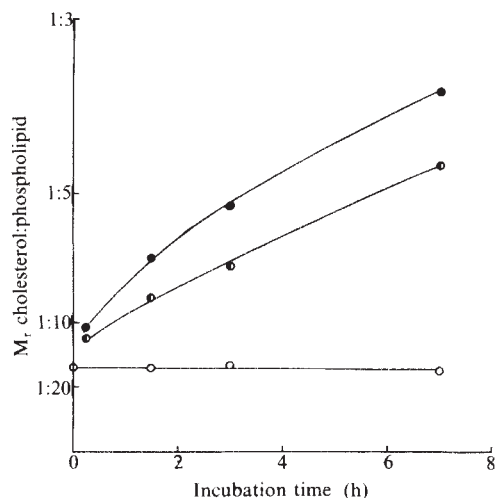


Fig. 1 Change in the ratio of cholesterol to phospholipid in SR membranes during incubation in the presence of cholesterol-rich liposomes. SR vesicles ($1.5 \text{ mg protein ml}^{-1}$) were incubated in the absence of liposomes (○), with $1 \text{ mg dipalmitoyllecithin-cholesterol per ml}$ (●), or $2 \text{ mg dipalmitoyllecithin-cholesterol per ml}$ (●) in a medium consisting of 0.1 M KCl , $5 \text{ mM histidine buffer pH } 7.4$, 100 U ml^{-1} penicillin-G and $100 \mu\text{g ml}^{-1}$ streptomycin sulphate. The incubations were performed in siliconised flasks gassed with nitrogen and shaken gently in a water bath maintained at 20°C .

shell of immobilised lipid, referred to as an annulus, which controls the enzyme activity. The shell is said to exclude cholesterol so that cholesterol molecules do not influence the enzyme activity. We report here the use of cholesterol-enriched liposomes to reversibly vary the content of cholesterol in the sarcoplasmic membranes. We show in contrast to the previous work that as the cholesterol content of the membrane varies so does the activity of the Ca^{2+} -ATPase.

Sarcoplasmic reticulum Ca^{2+} -ATPase is an example of an intrinsic protein where a single lipid shell of some 30 lipid molecules is bound relatively tightly to the protein. This immobilised layer or annulus is assumed to co-exist with and to be separate from the bulk lipid. The intrinsic protein is then envisaged to exist as a protein-lipid complex undergoing rotational and lateral diffusion within the remaining fluid lipid bilayer⁴. This has led to the suggestion that a similar situation occurs with a major class of other membrane proteins⁶. Methods of changing the cholesterol content of biomembrane systems *in vitro* have now been developed. Incubation of cholesterol-rich liposomes with a variety of cells and subcellular organelles has been shown to cause a rapid exchange of the sterol and there is a

net transfer of cholesterol on creation of a concentration gradient^{7,8}. We have exploited this system to alter the cholesterol content of sarcoplasmic reticulum (SR) by incubating membrane vesicles with cholesterol-rich liposomes. The effect of introducing cholesterol on the activity of Ca^{2+} -dependent ATPase in the natural biomembrane system was examined.

SR isolated from rabbit hind leg muscle has a relatively simple protein and lipid composition^{9,10}. More than 60% of the total membrane protein is Ca^{2+} -dependent ATPase which is responsible for pumping Ca^{2+} from the sarcoplasm into the cisternae of the SR during the recovery phase leading to muscle relaxation. Calcium uptake by SR vesicles has been shown to be stoichiometrically coupled to ATP hydrolysis^{11,12}. The membrane-bound enzyme has a molecular weight of about 110,000 and it is partly interpolated into the lipid bilayer¹³.

Preliminary experiments investigated the rate and extent of cholesterol incorporation into SR vesicles. SR, prepared by the method of Martonosi *et al.*¹⁴ and further purified by sucrose density gradient centrifugation¹⁵, was incubated with cholesterol-rich liposomes. These were prepared by co-lyophilising dipalmitoyllecithin and cholesterol (molar ratio (M_r), 1:2) from benzene:methanol 95:5 (v/v) and then sonicating the mixture in a sealed vessel under nitrogen for 12 min at 30°C in distilled water. After sonication, the liposome suspensions were centrifuged at $50,000g$ for 60 min to remove undispersed lipid and titanium dislodged from the sonicator probe. Aliquots of the incubation mixture were removed at appropriate time intervals and cholesterol-rich liposomes were separated from SR vesicles by the following procedure. The suspension was diluted with 2 vol. 0.1 M KCl , $5 \text{ mM histidine buffer pH } 7.4$, and centrifuged for 30 min at $50,000g$. The membrane fraction sedimenting as a pellet was resuspended in 20% (w/v) sucrose buffered to pH 7.4 with 5 mM histidine and layered at the interface between 27% sucrose, 5 mM histidine , pH 7.4 and 0.1 M KCl , 5 mM histidine , pH 7.4. Centrifugation of the gradient for 2 h at $100,000g$ separates any remaining cholesterol-rich liposomes. These band at the interface of 0.1 M KCl and 20% sucrose layers and the SR is recovered as a pellet at the bottom of the gradient.

Figure 1 shows changes in the M_r of cholesterol:phospholipid in the purified membrane fractions during incubation with cholesterol-rich liposomes. Cholesterol was extracted from the SR by the method of Rose and Oklander¹⁶ after saponification of the lipid, and assayed by quantitative gas chromatography using an internal standard of cholestane. The amount of cholesterol transferred to the membranes can be seen to vary both with the concentration of cholesterol-rich liposomes and with incubation time. No loss of phospholipid¹⁷ or protein¹⁸ from the SR could be detected and there was no incorporation of dipalmitoyllecithin (Table 1) into the membrane as judged from the pattern of fatty acid methyl esters separated by gas chromatography¹⁹. In separate control experiments, membrane vesicles were incubated with pure dipalmitoyllecithin liposomes which, in contrast to cholesterol-rich liposomes, could not be separated from the SR by the method described. Fusion of the cholesterol-rich liposomes with the membrane is excluded on the basis of freeze-fracture electron microscopy which shows two distinct populations of vesicles, one with prominent intramembrane particles, believed to represent intrinsic protein of the SR, and the other devoid of particles, presumably the phospholipid liposomes. Adhesion of liposomes to the vesicles did not effect Ca^{2+} -ATPase throughout the 7-h incubation.

The relationship between Ca^{2+} -dependent ATPase activity and the cholesterol content of the SR membrane is shown in Fig. 2. Calcium-dependent ATPase activities were measured at 32°C in the presence of an ATP regenerating enzyme system. The assay medium consisted of $0.1 \text{ M triethanolamine buffer pH } 7.4$, 0.1 M KCl , 5 mM MgCl_2 , 0.5 mM EGTA , 3 mM Mg-ATP , $1.25 \text{ mM phosphoenolpyruvate}$, 0.2 mM NADH , pyruvate kinase (20 IU) and lactate dehydrogenase (20 IU) in a total volume of 3 ml. The SR was preincubated in the assay medium for at least 10 min and the Ca^{2+} -independent ATPase activity determined: Ca^{2+} -dependent ATPase was then measured

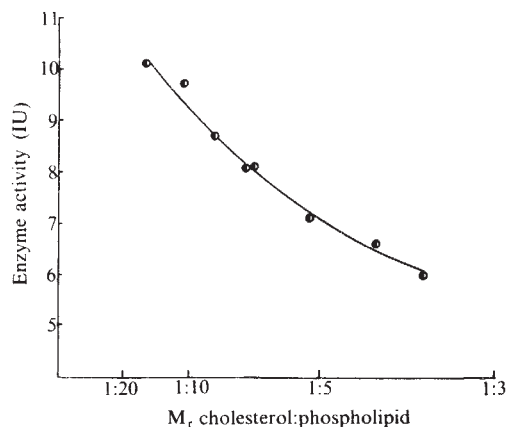


Fig. 2 Ca^{2+} -dependent ATPase activity of SR as a function of the ratio of phospholipid to cholesterol in the membrane. Enzyme assays were performed as described in the text.

Table 1 Analysis of SR vesicles before and after incubation

Sample	Concentrations of CRL in incubation	Incubation time (h)	Protein concentration (mg ml ⁻¹)		Phospholipid mg ml ⁻¹)		Protein/phosphilipid ratio			
	medium (mg ml ⁻¹)									
SR Vesicles, control	0	0	1.51 ± 0.02		1.07 ± 0.02		1.41 ± 0.02			
	0	7	1.48 ± 0.03		1.05 ± 0.02		1.41 ± 0.03			
SR cholesterol supplemented	1	0	1.49 ± 0.02		1.05 ± 0.01		1.42 ± 0.02			
	1	7	1.48 ± 0.03		1.06 ± 0.02		1.40 ± 0.03			
SR cholesterol supplemented	2	0	1.50 ± 0.02		1.07 ± 0.02		1.40 ± 0.02			
	2	7	1.48 ± 0.02		1.05 ± 0.02		1.41 ± 0.02			
Analysis of major fatty acids (% total)										
			16: alde	16:0	18:0	18:1	18:2	18:3	20:4	
SR control	0	7	4.0 ± 0.3	27.7 ± 0.5	12.8 ± 0.3	19.5 ± 0.5	24.5 ± 0.6	3.1 ± 0.3	8.4 ± 0.5	
SR cholesterol supplemented	2	7	4.1 ± 0.3	27.6 ± 0.6	13.1 ± 0.4	19.5 ± 0.4	24.8 ± 0.6	2.8 ± 0.3	8.4 ± 0.5	

Values given are mean ± s.e.m. for four samples. CRL, cholesterol-rich liposomes.

following addition of CaCl₂ to give a final assay concentration of 0.55 mM. The rate of oxidation of NADH was monitored on a recording spectrophotometer.

It can be seen that Ca²⁺-stimulated enzyme activity is decreased in direct proportion to the cholesterol content of the membrane over the range examined. Cholesterol is known to restrict the motion of disordered fatty acyl chains of pure phospholipid dispersions or phospholipids in biological membranes^{20,21}. This results in a reduction in enthalpy of the lipid thermotropic phase transition and a decrease in fluidity of membranes at temperatures above the phase transition temperature. Recent physical studies of SR membranes using high angle X-ray diffraction, NMR spectroscopy and calorimetric techniques have suggested that the lipids are all in a liquid-crystalline state^{22,23} above 10 °C. The effect of cholesterol on Ca²⁺-dependent ATPase is therefore consistent with an effect on the fluidity of the SR membrane.

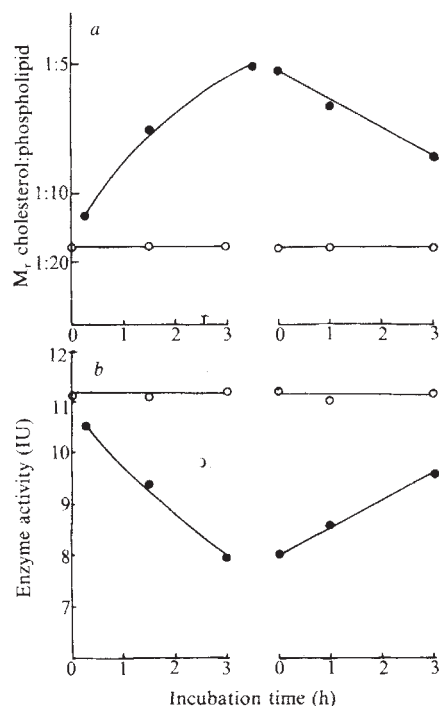


Fig. 3 Restoration of Ca²⁺-ATPase activity on removal of added cholesterol. SR (1.5 mg protein per ml) was incubated in the presence of 2 mg dipalmitoyllecithin-cholesterol per ml for 3 h followed by incubation with 1 mg of egg-yolk lecithin per ml for 3 h (●), or in the absence of liposomes (○). *a*, Cholesterol content of the membranes at intervals during incubation; *b*, corresponding Ca²⁺-ATPase activities.

To investigate this further and to discount the possibility that cholesterol interacts directly with the enzyme to cause an irreversible loss of activity, cholesterol was removed from cholesterol-supplemented membranes. Figure 3 shows results for a membrane preparation incubated with cholesterol-rich liposomes and subsequently with liposomes of egg-yolk lecithin to partially re-extract the incorporated cholesterol.

Qualitatively similar results were obtained when SR was incubated with lower liposome concentrations (1 mg ml⁻¹). Removal of cholesterol from the SR membranes results in a corresponding recovery of Ca²⁺-dependent ATPase activity. This confirms that cholesterol does not irreversibly inactivate the enzyme and that the presence of cholesterol in the membrane causes a modulation of the Ca²⁺-ATPase activity. The only interpretation possible for these results is that if an annulus as envisaged by Warren *et al.*⁵ does exist around this protein then cholesterol is not excluded. This is in contrast to the conclusion of these workers.

The existence of such an annulus has been recently questioned. Studies have shown that almost full enzymatic activity can be recovered after replacing virtually all of the membrane phospholipids with suitable detergents²⁴. Furthermore, biochemical²⁵ and biophysical evidence (for example ²H NMR and ESR spectroscopy)²⁶ is considered to be inconsistent with the assumptions inherent in the annulus concept.

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ATP induces nucleotide permeability in rat mast cells

ATP is one of several agonists which stimulate Ca^{2+} -dependent exocytotic degranulation of mast cells, causing the release of histamine and other mediators of immediate hypersensitivity^{1–3}. The concentration–effect relationship of ATP on mast cells is characterised by the sharp onset of self-inhibition as the concentration is raised above the optimum for histamine secretion⁴. We have previously shown that stimulation of mast cells with IgE-directed ligands (antigen, concanavalin A), chymotrypsin and compound 48/80 is accompanied by an increased turnover of phosphatidylinositol⁵ similar to that observed for Ca^{2+} -mediated responses in other tissues⁶. At concentrations of ATP which cause inhibition we have observed a reduction in phosphatidylinositol turnover to less than control levels (unpublished results). We show here that the inhibition by ATP is correlated with an extensive leakage of phosphorylated metabolites from the cells.

The effective agonist form of ATP is ATP^{4-} (refs 3, 4); that is, the ATP which remains unbound to the divalent cations Mg^{2+} and Ca^{2+} , which are normal components of physiological salt solutions. Optimal concentration for ATP^{4-} -mediated secretion is about $3\text{ }\mu\text{M}$. With ATP^{4-} applied at $5\text{ }\mu\text{M}$, inhibition of secretion due to itself⁴ or other ligands is almost total⁷ and a parallel inhibition of glycolytic metabolism has also been observed⁸. Rat peritoneal mast cells were preincubated with ^{32}P -phosphate, washed free of external radioactivity and purified to better than 95% homogeneity (see Fig. 1 legend). When we applied ATP to these cells at a concentration optimal for secretion (Fig. 1a) (at which ATP^{4-} was calculated to be $2.9\text{ }\mu\text{M}$), we found that there was an increased efflux of ^{32}P -labelled material from the cells (Fig. 1b). As the concentration of free ATP was raised to $8.6\text{ }\mu\text{M}$, histamine secretion was prevented (Fig. 1a) but the loss of ^{32}P -labelled material increased (Fig. 1b). ATP applied to the cells at concentrations below the optimum for secretion had only a small effect on the loss of label (Fig. 1a, b).

We separated the extracellular material into three fractions by passage through activated carbon (to separate nucleotides)⁹ and by extraction into isopropylacetate, after formation of the phosphomolybdate complex to separate inorganic phosphate from organic phosphates (probably phosphorylated intermediates of glycolytic metabolism)¹⁰. Figure 2 shows the appearance of radioactivity in these three fractions as functions of ATP concentration and time.

At low concentrations ($1.4\text{ }\mu\text{M}$ free ATP, sub-optimal for secretion) the labelled material appearing outside the cells is entirely in the form of inorganic phosphate. As the concentration of free ATP is raised through $2.9\text{ }\mu\text{M}$ (optimal for secretion) to $8.6\text{ }\mu\text{M}$ (inhibitory) the rate of loss of inorganic phosphate from the cells increases, but in these conditions a considerable loss of nucleotides and other ^{32}P -labelled material also occurs. This loss of metabolites is not a consequence of exocytotic degranulation, as extensive histamine secretion due to application of compound 48/80 is not accompanied by the appearance of labelled metabolites in the extracellular medium.

Loss of metabolites on this scale would be quite sufficient to account for the inhibitory effects of externally applied ATP on glycolysis⁸ and the ATP-induced phosphatidylinositol response. Exogenous ATP is known to permit the leakage of intracellular anions (including nucleotides) from some transformed (but not

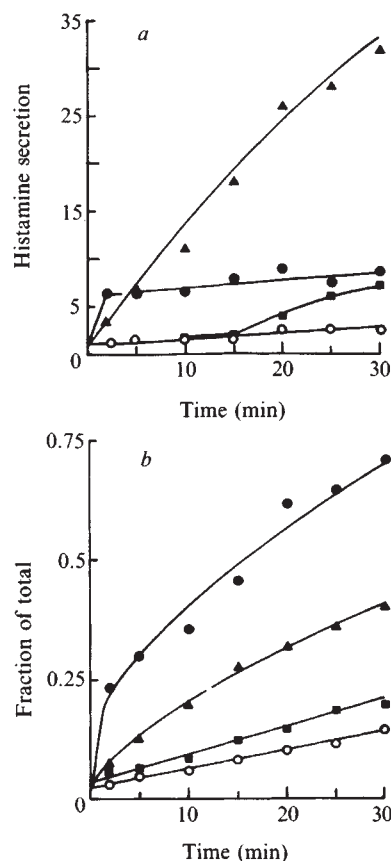


Fig. 1 *a*, Time course of histamine secretion from purified rat peritoneal mast cells, induced by various concentrations of ATP. *b*, Time course of ATP-induced loss of ^{32}P -labelled metabolites from mast cells. Rat peritoneal cells were obtained by peritoneal lavage and suspended in 2 ml of a balanced salt solution (composition previously described⁵). The mixed cells were loaded with ^{32}P - PO_4 ($100\text{ }\mu\text{Ci ml}^{-1}$, carrier-free) by incubation at 37°C for 2 h. The mast cells were then isolated from the extracellular ^{32}P - PO_4 and purified to >95% homogeneity by centrifugation (400g, 15 min) through a cushion of Ficoll (30%) as described elsewhere⁵. The purified mast cells were then washed twice by centrifugation and suspended in the balanced salt solution at 5×10^5 cells per ml. The cells were incubated at 37°C with concentrations of ATP as indicated. At various times 250 μl were removed and quenched into 1 ml of ice-cold saline. After centrifugation at 4°C (400g, 5 min) samples of supernatant were removed for measurement of radioactivity and of histamine (method as previously described¹⁷). Both the total and free ATP concentrations are indicated; free ATP concentrations were calculated from equilibrium considerations with $K_{(\text{Mg},\text{ATP})} = 10^{4.28}$ and $K_{(\text{Ca},\text{ATP})} = 10^{3.94}$ (ref. 4). ATP concentration (μM) (total/free): $\circ$, 0/0; $\blacksquare$, 50/1.4; $\blacktriangle$, 100/2.9; $\bullet$, 300/8.6.

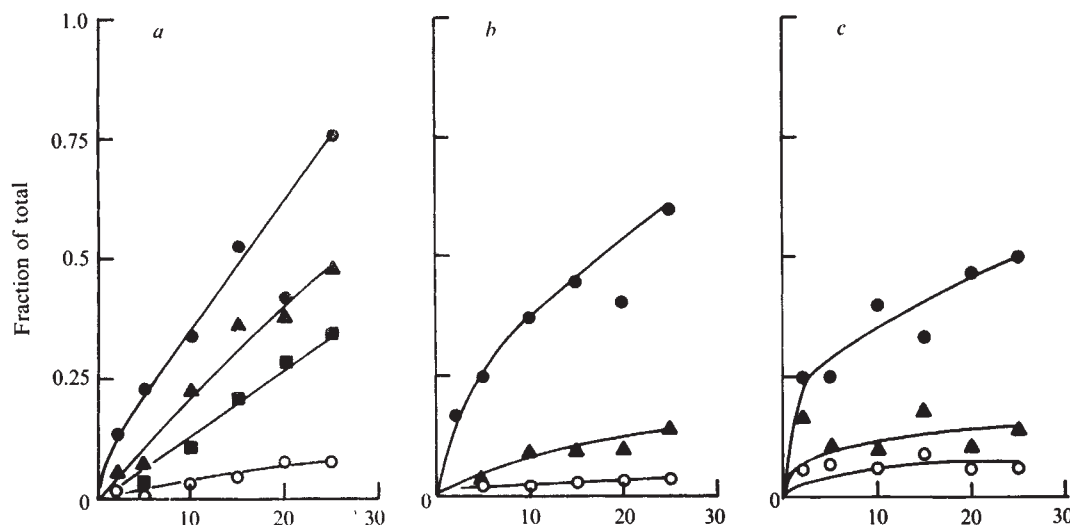


Fig. 2 Time course of ATP-induced loss of inorganic phosphate (a), non-nucleotide organic phosphates (such as sugar phosphates) (b), and nucleotides (c), from prelabeled mast cells. After incubation for various times in the presence of ATP, the supernatants were collected as described in Fig. 1, and samples fractionated as described in the text. Free ATP concentration (μM): $\bullet$, 8.6; $\blacktriangle$, 2.9; $\blacksquare$, 1.4; $\circ$, 0.

untransformed) cell lines¹¹, but we stress that this effect of ATP occurs at concentrations more than two orders of magnitude above that which causes leakage of metabolites from mast cells. The effect on mast cells shows specificity for ATP; 2-deoxy ATP showed a small effect when applied at 500 μM (total concentration), and CTP, GTP, ADP, adenosine and the synthetic ATP analogue, adenylylimidodiphosphate, were without effect when applied at 1 mM (total); they did not prevent the action of ATP.

The affinity of the ATP receptor on mast cells is of the order of 10^6 l mol^{-1} but this might be modulated by the presence of divalent cations. In the absence of divalent cations nucleotide loss from mast cells began with ATP⁴⁻ applied at 1 μM and was maximal with ATP⁴⁻ at 3 μM , conditions which permit only the loss of inorganic phosphate in the presence of Mg^{2+} and Ca^{2+} (see Fig. 2). The divalent cations which were present in our experiments to act as buffers for the ATP and to permit secretion may thus additionally confer protection on the cells against the detrimental effects of ATP. We cannot say whether the ATP receptor responsible for enhanced anion permeability is the same as that which stimulates phosphatidylinositol turnover, Ca^{2+} and Na^{+} fluxes¹² and histamine secretion. The enhanced permeability does not extend to macromolecules (leakage of lactate dehydrogenase was less than 5%) and is in no sense lytic.

Perhaps the most interesting observation is the graded response to molecules of increasing molecular weight as the concentration of ATP applied to the cells is increased. Thus, free ATP at 1.4 μM enhances the permeability of the mast cells only to inorganic phosphate, which increases as the concentration of free ATP is raised to 2.9 μM . It is only above this level that sugar phosphates and nucleotides begin to appear. This indicates that as the concentration of external ATP is raised, a change occurs in the filtration properties of the anion channels through which the intracellular metabolites emerge. One possibility is the successive involvement of channels of different specificity; alternatively, it would result from variation in the dimensions of a single channel type. A possible mechanism would involve channel formation by the aggregation of transmembrane monomeric units in the manner which has been discussed with respect to the channels formed by the polyene antibiotics¹³ and alamethicin¹⁴. In the latter case it has been argued that enlargement of pre-formed channels occurs by the recruitment of additional monomers into the oligomeric conducting unit. The conductivity of the junctional membrane channels of *Chironomus* salivary gland varies inversely with the concentration of intracellular Ca^{2+} (ref. 15) and this correlates with variation in the size exclusion limits for transfer of a series of fluorescent dyes between neighbouring cells¹⁶. In this case, too, it has been suggested that the variable filtration characteristics could arise from alterations in effective channel size.

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Choline transport is not coupled to acetylcholine synthesis

It has been proposed^{1–3} that choline transport, particularly that performed by the so-called high-affinity system, is obligatorily coupled to acetylcholine synthesis and therefore only choline that has just entered by the transport mechanism is acetylated. If this were so it would imply that the enzyme choline acetyltransferase is bound close to the transport sites on the membrane. The problem is important because of its implications for the molecular organisation of the synapse and also because subcellular fractionation studies^{4,5} show fairly clearly that, at the ionic strengths prevailing inside the cell, choline acetyltransferase is soluble and not bound to the plasma or synaptic vesicle membrane (as would be expected if the hypothesis of obligatory coupling is true). The evidence for this is indirect and relies on the finding that the half-maximal concentration for acetylation of choline and its analogues *in situ* approximates more nearly to the half-maximal concentration of their transport than of their acetylation by the isolated enzyme¹. However, this

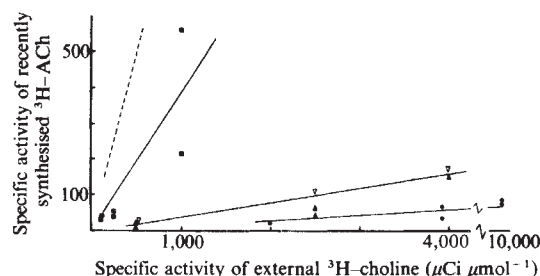


Fig. 1 Utilisation of external choline for ACh synthesis. Synaptosomes were preincubated as described in the text with 8 μM ^{14}C -glucose (304 $\mu\text{Ci } \mu\text{mol}^{-1}$) and then washed and incubated for 10 min with different concentrations (●, 0.2 μM ; ▲, 0.8 μM ; ■, 10 μM) of choline at various specific activities. Dashed line shows theoretical 100% curve. Each point is the result of separate incubation. ▽, Incubation carried out in 24 mM K^+ which depolarises and causes ACh release. Ordinate, specific activity of the ^3H -ACh synthesised during the 10 min incubation with ^3H -choline. ^{14}C -ACh present in synaptosomes just before the addition of the pulse was 533 ± 56 d.p.m. per mg (s.e.m., $n = 10$) and rose to 917 ± 28 d.p.m. per mg (s.e.m., $n = 20$) during the 10-min choline pulse.

need only imply a coupling between transport and synthesis if the latter is rate controlling, and from a survey⁶ of the rates of release and synthesis of acetylcholine (ACh) it seems that the activity of choline acetyltransferase is in considerable excess of normal requirements. Certain exceptions to the obligatory coupling of transport and synthesis have been noted but these involve synthetic analogues of choline⁷ or unusual preparations^{8,9}. Reduction of the acetyl-CoA levels reduces ACh concentrations without a concomitant reduction in choline transport¹⁰ but neither this nor the previous studies exclude the possibility that the residual ACh synthesis remains coupled; that is, obligatorily dependent on choline that has just been transported across the membrane. We describe here an experiment which we believe demonstrates that choline that has been recently transported has no privileged access to choline acetyltransferase in the synaptic terminal. Instead it mixes with the pre-existing pool of choline and therefore there is no coupling between transport and synthesis.

The principle of the experiment is that if there was obligatory coupling only choline originally outside the synaptic terminal would be acetylated. If, therefore, the terminals are exposed to pulses of choline of different specific ^3H -radioactivities, the specific activity of the choline moiety of ACh synthesised inside the terminal during the pulse should be exactly that of the choline to which the terminals were exposed. Quantitatively, a plot of the specific activity of the recently synthesised ACh

against the specific activity of the choline pulse will have a slope numerically equivalent to its contribution towards ACh synthesis. This will be unity if coupling is obligatory, and will decrease as the contribution of the transported choline to the total amount of choline utilised decreases.

To distinguish recently synthesised ACh from pre-existing stores and estimate its specific radioactivity, acetyl CoA was labelled by pre-incubation of synaptosomes with ^{14}C -glucose. The specific ^{14}C radioactivity of the ACh synthesised during the ^3H -choline pulse will be identical to the specific ^{14}C radioactivity of the acetyl CoA during the period of the pulse. As specific activities are ratios of radioactivity to moles the denominators cancel and the specific ^3H radioactivity of the recently synthesised ACh is given by the ratio of ^3H - to ^{14}C -labelled ACh synthesised during the pulse, multiplied by the specific ^{14}C radioactivity of the acetyl CoA.

Nerve terminals from cerebral cortex (synaptosomes) were used because although they are heterogenous with respect to the transmitter, their metabolic characteristics are understood better than alternative preparations of cholinergic synaptosomes. The characteristics of carrier-mediated choline transport in these particles compare favourable with that in intact tissues¹¹. The synaptosomes were isolated by conventional procedures, introduced into and washed twice with a physiological saline containing 180 mM NaCl, 3 mM KCl, 2 mM CaCl_2 , 2 mM MgCl_2 and 8 mM NaPi (pH 7.2) but without glucose. ^{14}C -glucose (8.2 μM , 304 $\mu\text{Ci } \mu\text{mol}^{-1}$) was then added and the synaptosomes incubated at 37 °C for 60 min to label the acetyl CoA. The low concentration of glucose was used to achieve a satisfactory incorporation of radioactivity into acetyl CoA with reasonable economy of isotope. Some experiments were carried out at a glucose concentration of 0.5 mM with similar results. These and a comparison of the uptake of ^3H -choline and conversion to ^3H -ACh at higher glucose concentrations are given in Table 1.

After the incubation an aliquot of the suspension was taken to determine the initial value of ^{14}C -ACh. The ^3H -choline pulse was then added so that the final concentrations of choline were 0.2, 0.8 and 10 μM and at each concentration there were three different specific activities in the range 50–10,000 $\mu\text{Ci } \mu\text{mol}^{-1}$. After 10 min the synaptosomes were sedimented by a rapid (5-s) centrifugation in a Janetski centrifuge and the washed pellets taken for analysis. ^3H and ^{14}C radioactivities in ACh were determined after extraction and thin layer chromatography¹². The specific radioactivity of the ^{14}C -acetyl CoA was measured after trichloroacetic acid extraction by removing the choline with two washes of sodium tetraphenylboron (10 mg ml^{-1}) in vinyl acetonitrile and then allowing the acetyl CoA to acetylate enzymatically radioactive ^3H -choline of known specific activity using the choline acetylase of a brain acetone powder. The

Table 1 Utilisation of external choline for acetylcholine synthesis in various conditions

Choline concentration (μM)	Glucose concentration (mM)	Influx of ^3H -choline in 10 min (pmol per mg)	Synthesis of ^3H -ACh in 10 min (pmol per mg)	Fractional utilisation of external choline $\pm$ s.e.m. (n)	Influx of ^3H -choline in 10 min expressed as a fraction of total endogenous choline
0.2	0.008		2.0	0.013	
0.2	0.5	7.6	2.0	0.031 ± 0.06 (4)	0.02
0.2	10.0	8.0	4.9		
0.5	0.5		5.5	0.039 ± 0.001 (4)	
0.5 (depolarising medium)	0.5			0.048	
0.5	10.0	16.0	9.5		0.05
0.8	0.008			0.029	
0.8 (depolarising medium)	0.008			0.038	
10	0.008		36	0.360	
10	0.5	79	50	0.485 ± 0.029 (4)	0.2
10	10.0	90	51		

The influx of ^3H -choline and synthesis of ^3H -ACh were measured at the various choline and glucose concentrations in separate experiments¹⁴ and are expressed as pmol per mg synaptosomal protein. The fractional utilisation of external choline was calculated from the slopes of plots made as in Fig. 1. In the last column the influx of choline in 10 min is expressed as fraction of the total synaptosomal content of endogenous choline determined in separate experiments¹⁴ as 400 pmol per mg synaptosomal protein.

specific ^{14}C radioactivity of the synaptosomal acetyl CoA was $2.2 \mu\text{Ci } \mu\text{mol}^{-1}$ and it did not change significantly during the pulse.

The results for a typical experiment are shown in Fig. 1. As expected the points fall on straight lines passing through the origin. Duplicate data show reasonable agreement. Incubating the synaptosomes in a depolarising medium¹³ so that they released ACh had little effect. The slopes are much less than unity at choline concentrations of 0.2–0.8 μM , but increase to about 0.5 at choline concentrations of 10 μM . This means that for the synthesis of ACh there is a substantial dilution of the external choline by the choline pre-existing in the terminal, and therefore there does not seem to be any coupling of transport to synthesis. Furthermore as the choline concentration was increased out of the 'high-affinity' range the slopes increased, indicating a greater utilisation of external choline. This is the opposite to what would be expected if the high-affinity uptake alone was coupled to ACh synthesis, as at higher choline concentrations its effect would be swamped by the low-affinity uptake. In fact the utilisation of external choline agrees tolerably well with the contribution of influx to the total synaptosomal choline pool, as can be seen from the summary of results in column 6 of Table 1.

Synaptosomes derived from cerebral cortex are not all cholinergic but this will not affect our results as non-cholinergic synaptosomes do not synthesise ACh and will therefore not have contributed to the data. We have considered the possibility that efflux of choline from the synaptosomes was diluting the ^3H -choline in the medium. Choline efflux was measured and although some dilution occurred the qualitative aspects of the experiment were preserved. The uptake of ^3H -choline and its conversion to ^3H -ACh are slightly but not markedly reduced (Table 1, columns 3 and 4) by the low concentrations of glucose used in this study. It could be argued that, even though these are relatively unaffected by low glucose concentrations, there might be interference with other aspects of synaptosomal functioning that indirectly affect the results. However, the overall pattern of the coupling is maintained at a glucose concentration differing by 20-fold (that is from 8 μM to 0.5 mM) so it seems reasonable to suppose that this pattern will be preserved at the 2–10-fold higher concentration of glucose conventionally used.

Although these experiments were not designed to demonstrate the existence or otherwise of high-affinity transport systems specifically associated with cholinergic synaptosomes^{15–18} they do not support this notion. If there was such a system it would follow that the proportional utilisation of external choline at low concentrations would exceed the utilisation at high concentrations. The reverse is the case; moreover the contribution of transport (measured over the whole population) to the total synaptosomal choline pool is in reasonable agreement with its contribution to ACh synthesis in cholinergic synaptosomes. High-affinity uptake, if it exists, makes only a minor contribution to the synthesis of the transmitter.

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Effects of catecholamines, ATP and ionophore A23187 on potassium and calcium movements in isolated hepatocytes

IN addition to their well known effects on hepatic gluconeogenesis and glycogenolysis, adrenaline and noradrenaline cause a transient net loss of potassium from the liver of many species¹. Studies with guinea pig and rabbit liver slices have shown this to be a consequence of a predominantly α -adrenoceptor-mediated increase in the potassium permeability (P_K) of the hepatocyte membrane^{2–4}. It has recently become possible to isolate hepatocytes in high yield and with cation levels and metabolic capabilities superior to those of slices, and approaching that of the intact tissue⁵. We have now confirmed that the effects of catecholamines on potassium movement can be shown with such cells, and we have obtained evidence to support the suggestion that the potassium loss which follows α -adrenoceptor activation in guinea pig liver cells is a consequence of an increase in potassium permeability triggered by a rise in intracellular calcium. The mechanism can be blocked by quinine, as in human erythrocytes¹⁹ and barnacle photoreceptors²⁰.

Haylett⁴ first raised the possibility that the increase in P_K in liver cells is a consequence of a rise in the concentration of calcium ions in the cytosol (see also refs 6, 7). Evidence for the two processes envisaged in this proposal, an effect of α -receptors on intracellular calcium, and control of P_K by cytosolic calcium, has been obtained in various tissues including salivary and lacrimal glands^{6–12}. On such a scheme we could expect the divalent cation ionophore A23187 to initiate potassium loss by increasing cytosolic calcium, and hence P_K . This was tested with hepatocytes isolated by Seglen's method^{13,14} from the liver of male Hartley guinea-pigs (220–300 g). The cells were equilibrated at 37 °C for 30–60 min in Eagle's medium¹⁵ (Wellcome) containing (mM): NaCl, 116; KCl, 5.4; CaCl_2 , 1.8; MgSO_4 , 0.81; NaH_2PO_4 , 0.96; NaHCO_3 , 25, and (mg l^{-1}): amino acids, 805; vitamins, 8.1; glucose, 1,000; L-glutamine, 292; phenol red, 10. This was supplemented with 2% albumin (fraction V, Sigma). The pH was maintained at 7.4 by circulating 5% CO_2 in O_2 above the cell suspension which was shaken at 120 strokes per min. In some experiments (including those with ^{45}Ca), samples of the suspension were filtered to eliminate the largest clumps of cells and then centrifuged at 50g for 2 min. The cell pellet was resuspended either in normal Eagle's medium or in a low-Ca, low-Mg variant (Ca, 0.1 mM; Mg, 0.05 mM) sometimes containing ^{45}Ca (0.2 $\mu\text{Ci ml}^{-1}$) but without albumin. The viability of the cells was checked before and after each experiment by their ability to exclude Trypan blue (4%). By this test between 80 and 85% of the cells were viable at the time when drugs were applied.

Figure 1 shows that A23187 caused a rapid loss of potassium from the isolated cells, as did the α -agonists amidephrine^{16,17}, phenylephrine (not illustrated) and noradrenaline. ATP was also found to be effective. This is in keeping with the earlier observation¹⁸ that ATP hyperpolarises cells in guinea pig liver slices, presumably by increasing P_K . The potassium loss was not associated with a change in cell viability. It was greatest with A23187, reaching a maximum of $23 \pm 2\%$ (s.e., $n = 8$) of the

initial cell content (about 290 mmol per kg dry weight) within 1 min when the ionophore was applied at 40–80 μM per litre of cells, corresponding to an added concentration of 1 μM . With maximal doses of ATP and noradrenaline (100 and 5 μM respectively) the loss was 10% with each agent, again within about a minute. This is of the same order (8%) as seen on exposing guinea pig liver slices to 1 μM noradrenaline². The response to the selective α -agonist amidephrine was somewhat variable, ranging from 7% to as little as 1% in a few preparations.

The next experiments were prompted by the finding of Armando-Hardy *et al.*¹⁹ that in erythrocytes the influence of cytosolic calcium on P_K is inhibited by quinine. A similar effect has been observed in barnacle photoreceptors²⁰. We might then expect this agent to reduce the effectiveness of the ionophore and of the other agents in inducing K loss from liver cells. Figure 1a shows that this is so; the responses to noradrenaline and amidephrine were also inhibited to a comparable extent. The possibility that quinine was acting by interfering with the primary actions of the ionophore (and, by implication, of the other agents causing potassium loss) was tested by examining the effect of quinine on the ^{45}Ca movements brought about by A23187. The technique used was based on that described for erythrocytes by Ferreira and Lew²¹, and is described in Fig. 2. This illustrates the effect of A23187 on the ^{45}Ca content of cells equilibrated with the tracer for 30–40 min, long enough to allow most of the cytosolic calcium to exchange²². When the external

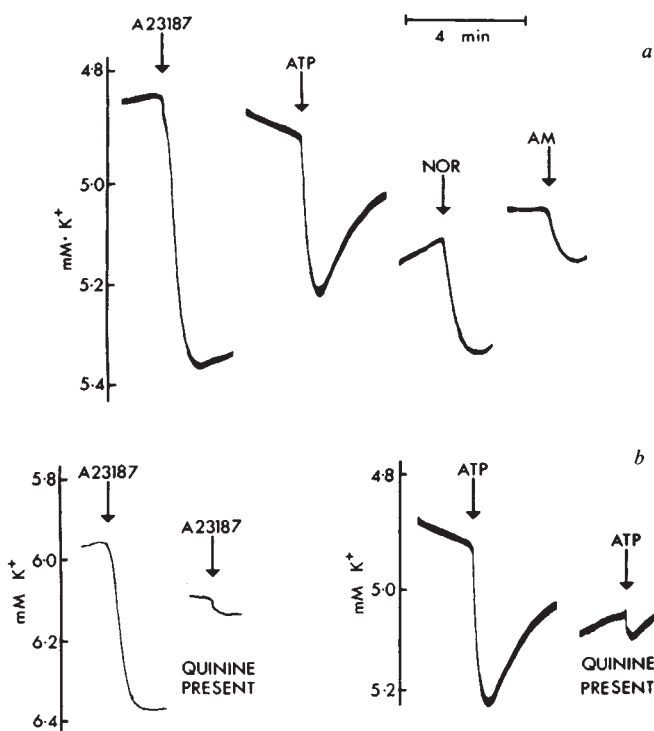


Fig. 1 a, Application of A23187 (5 μM , about $5 \times$ the dose for maximum effect), ATP (100 μM), noradrenaline (NOR; 5 μM in the presence of propranolol at 5 μM), and of the selective α -agonist (–) amidephrine (AM 10 μM) causes isolated guinea pig hepatocytes to lose potassium. The movement of K between the cells and the incubation medium (Ca, 1.8 mM; Mg, 0.81 mM) was followed by means of a K-sensitive electrode²⁹ in the cell suspension (which contained ~ 50 mg cells in 2 ml, and was stirred). A downward deflection indicates an increase in K concentration, corresponding to K loss from the cells: in this experiment, the loss in response to A23187 amounted to 19% of the total cell content. b, Quinine (1 mM, applied in 20 μl ethanol, 5 min previously) greatly reduces the K loss initiated by A23187 and by ATP: control responses are from the same batch of cells.

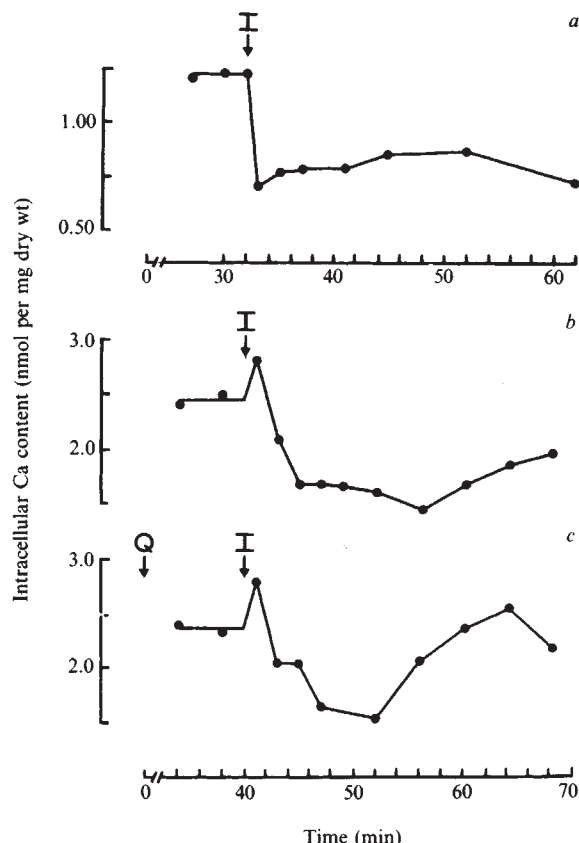


Fig. 2 Effect of A23187 on the ^{45}Ca content (nmol per mg dry weight) of guinea pig hepatocytes at two calcium concentrations (a, 100 μM , b and c, 1.8 mM). ^{45}Ca was included in the suspension fluid from zero time onwards. At the times indicated by the points, 100 μl samples of the cell suspension were placed in 1.5 ml Eppendorf tubes containing 300 μl of *n*-butylphthalate and 200–800 μl of 'washing medium'. This contained NaCl (160 mM), EGTA-NaOH (2 mM, at pH 7.4, to remove extracellularly bound ^{45}Ca) and ^3H -inulin, and was maintained at 0–1 $^{\circ}\text{C}$. Each sample was immediately centrifuged at 12,000g (Eppendorf centrifuge, Model 5412). The supernatant and the oil were sucked off, and the pellet of cells homogenised in 100 μl distilled water. ^{45}Ca and ^3H -inulin were measured by liquid scintillation counting. The amount of labelled inulin gave an estimate of the extracellular fluid trapped in the pellet, and this was used to calculate the ^{45}Ca content of the cells. A23187 was added (at I) in 20–70 μl absolute alcohol to 15 ml of the cell suspensions. (Final A23187 concentrations: a, 10 μM , 690 μmol per litre of cells; b and c, 2.7 μM , 82 μmol per litre of cells.) b and c are from the same experiment: in c, quinine was added in 15 μl ethanol to a final concentration of 1 mM. Note that quinine does not alter the first two phases of the ^{45}Ca movement caused by the ionophore.

calcium concentration was 1.8 mM (Fig. 2b), the ionophore first caused the ^{45}Ca content of the cells to increase. This occurred during the initial 15–60 s and was probably a consequence of a net calcium influx generated by the ionophore. A period of calcium extrusion then followed, during which the ^{45}Ca content fell to below the pre-ionophore level. This second phase has already been reported for rat liver cells^{23,24}, as well as for several other tissues^{25–28}, and has been attributed to the extrusion from the cells of ^{45}Ca released from intracellular stores which in spermatozoa have been localised to the mitochondria²⁶. In keeping with the idea that internal stores are concerned in liver cells, this phase (and the effect on potassium loss) was unaffected when the external calcium was reduced to 100 μM (Fig. 2a). However, the initial increase in ^{45}Ca could then no longer be distinguished, even when the concentration of ionophore was drastically increased (see Fig. 2a). This was presumably because the initial influx of calcium initiated by the ionophore was much

smaller. In high external calcium a third phase of ^{45}Ca re-accumulation was evident 10–30 min after the ionophore was applied. It was quite variable in both rate and magnitude and will not be discussed here since it occurs at a time when the potassium movements were largely complete.

Figure 2c shows that quinine at the same concentration as had greatly reduced the effects on potassium loss did not interfere with the first two phases of the action of A23187 on calcium exchange. Thus inhibition by quinine of the potassium movement must occur at a later stage, and would seem to be due either to uncoupling of the mechanism by which P_K is influenced by intracellular calcium, or to blockade of the potassium channels.

A surprising additional observation was that the rapid phase of potassium loss so evident on treating guinea pig hepatocytes with A23187, ATP and α -agonists was lacking in rat liver cells prepared in exactly the same way. With the rat cells A23187 caused a slow loss of potassium which became appreciable only after several minutes, that is, at a time when the rapid release from guinea pig cells was almost complete. This was not because of a reduced effectiveness of the ionophore since the effect of A23187 on calcium exchange was as great in rat as in guinea pig cells. These findings suggest that the calcium-sensitive potassium channel present in guinea pig liver cells (and, by implication, in those of the toad, rabbit, cat and dog, all of which show hyperkalaemia with adrenaline) is either lacking in rat hepatocytes, or exceptionally insensitive to calcium. That a species difference exists in this regard has been suspected^{1,6}, although it is surprising that it should be so striking.

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Enhancement of Ca spikes in nerve cells of adult mammals during neurite growth in tissue culture

GENERATION of Na and Ca spikes in various types of vertebrate nerve cells^{1–6} indicates that both Na and Ca channels are generally involved in inducing action potentials in their cell bodies. Although it has been suggested that these ionic channels may change their configuration in the plasma membrane, in particular during degeneration of nerve cells^{7,8} or during growth of axons associated with ontogenetic differentiation^{9–11}, no systematic or quantitative study has been reported. We have recently established a technique to obtain primary culture of nerve cells of mature mammals^{12,13}, and have also developed a technique to compare membrane capability of carrying inward Na and Ca currents among nerve cells of various ages in the cell culture. We report here that the capability of membranes to carry Ca currents, as represented by the maximum rate of rise (MRR) of a Ca spike, is enhanced during a particular period of the cell culture, whereas there is no significant change in Na currents. This may be explained as an increase in number of the Ca channels or as a change in conformation of the Ca channels, associated, in part, with neurite outgrowth. As we used nerve cells which had fully differentiated in the host bodies, the possibility of an effect due to ontogenetic differentiation in the tissue culture condition^{14–19} can be excluded. The present finding also demonstrates another aspect of nerve cell membrane that ionic channel molecules, especially the Ca channels, can alter their features rather flexibly in the plasma membrane.

Dorsal root ganglia (Th₁₂ to L₅) were dissected from adult guinea pigs (200–300 g body weight) in a sterile condition, and their nerve cells were dissociated by vibrating the ganglia in collagenase-containing Gey's medium. The nerve cells were collected by weak centrifugation, 1,500 r.p.m. for 5 min. The nerve cells thus obtained (Fig. 1a) were incubated at 37°C in collagen-coated 3-cm diameter plastic dishes (Nunc, approximately one ganglion per dish) with the tissue culture medium and with sterile air containing 5% CO₂. The tissue culture medium contained 75% Eagle's minimum essential medium (Microbiological Associates), 15% horse serum (Microbiological Associates) inactivated by heat (60°C for 1 h), 9% chick embryo extract and 1% penicillin-streptomycin solution (Flow). The medium was changed two or three times a week. So as to obtain steady and compatible cell culture, we carefully kept the cell culture condition uniform, in particular by using the same lot number of dishes, collagen, drugs and some medium components^{12,13}.

During the incubation, the nerve cells became attached to the bottom of the dishes in a few hours and then began to extend their neurites gradually in several directions (Fig. 1b). Active growth of the neurites was seen (Fig. 1c) during the first week of cell culture. After about 1 week in the cell culture, fibroblasts began to grow rapidly in number, and occupied most of the available areas of the bottom of the dishes in a few weeks; thus it often became difficult to see the neurites under the phase contrast microscope. The nerve cells, however, survived in the cell culture for more than 2 months^{12,13}.

The nerve cell soma was penetrated carefully by a glass microelectrode (20–30 mΩ, filled with 3 M KCl solution) under direct view by inverted phase contrast microscope. Action potentials were elicited in the nerve cells by intracellular passage of current through the recording microelectrode; the membrane potential was determined using a Wheatstone bridge balance. The MRR of the action potential, which was presumed to be proportional to a transient inward membrane current²⁰, was measured using an electronic differentiator. The electrophysiological study was done at room temperature, 26–28°C; pH of

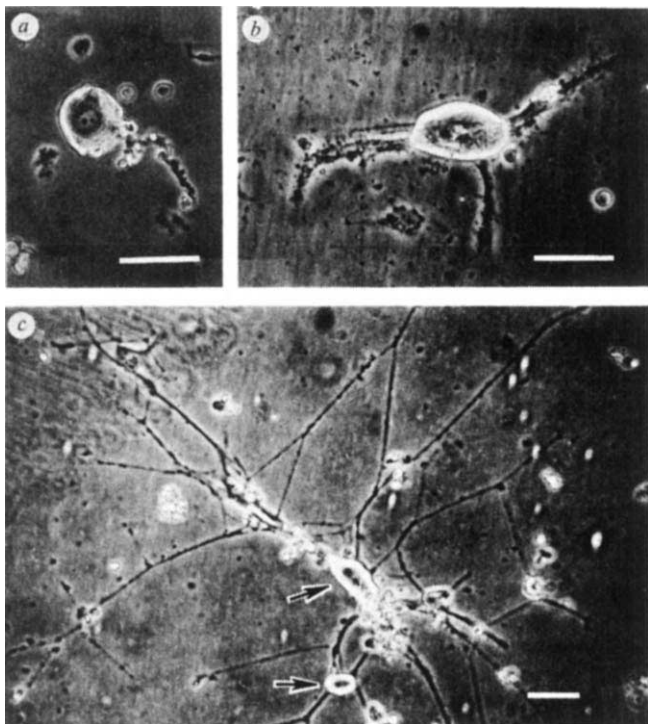


Fig. 1 Growth of dorsal root ganglion cells of adult guinea pigs in cell culture. *a*, A nerve cell immediately after being dissociated from a ganglion by collagenase treatment. *b*, A nerve cell after 2 days in cell culture. Neurite outgrowth is seen in several directions. *c*, Nerve cells (arrows) after 5 d in cell culture. The length of neurites was often more than 1 mm at this period of the nerve cell culture. Scale bar, 50 μm .

the medium was around 7.4 and was monitored continuously by phenol red.

Figure 2*a* and *b* illustrate spikes elicited in a nerve cell at the resting membrane potential (*a*) and at a hyperpolarised membrane potential (*b*). Judging from their MRRs (arrows in the lower traces), which were proportional to inward membrane currents associated with the respective spikes²⁰, the capability of inducing an inward transmembrane current was reduced in the resting membrane (for example, ref. 17) due to a rather small intracellular potential (usually between -45 mV and -65 mV). This inactivation was, however, removed by steady hyperpolarisation of the membrane potential below -80 mV (Fig. 2*b*: by d.c. current injection), where the MRR became steady at a maximal value which was independent of the membrane potential (Fig. 2*e*, open symbols). We considered this steady maximal value of the MRR as an indicator of the membrane capability of carrying inward current.

This inward current was carried by Na^+ as the spike generation was blocked either by externally added tetrodotoxin (TTX, $1.5\text{ }\mu\text{M}$) or by total replacement of external Na^+ with Tris^+ (data not shown). Although Ca current was possibly elicited in superimposing the spike potential, its contribution to the MRR above seemed to be very small, because the MRR was obtained within 1 ms after the spike onset (Fig. 2*a, b*); this delay was too short to elicit a large Ca current in various excitable membranes²¹⁻²³. Furthermore, the external growth medium contained a small amount of Ca^{2+} ($\sim 1.9\text{ mM}$) and no blocker of membrane K conductance (see below), which was not sufficient to induce a large inward Ca current in the nerve cell membrane.

A Ca spike (Fig. 2*c*) was elicited in a nerve cell bathed in a Na-free, tetraethylammonium (TEA)-containing medium; TEA^+ was added to minimise outward K current^{24,25}, so that a small inward current could induce regenerative development of the membrane depolarisation. In addition, 10 mM Ca^{2+} was

added externally to stabilise intracellular recording and to elicit a large spike potential. We used the Na-free medium described above instead of TTX-containing Na-rich medium because a part of the Na current elicited in tissue-cultured nerve cells is resistant to TTX¹⁰⁻¹³. We considered spikes elicited in the Na-free medium to be due to an inward Ca current for the following reasons²⁶: (1) their MRRs were related to external Ca concentration ($2.5\text{--}10\text{ mM}$); (2) they were not blocked by TTX high concentrations ($3\text{ }\mu\text{M}$)^{12,13}; (3) similar spikes were elicited in Na-free, Ba- or Sr-containing medium^{12,13} (10 mM , instead of Ca^{2+}); and (4), addition of Mg^{2+} (40 mM) or Co^{2+} (4 mM) suppressed the spike generation. Although a part of the Ca channel activity was blocked at a small resting membrane

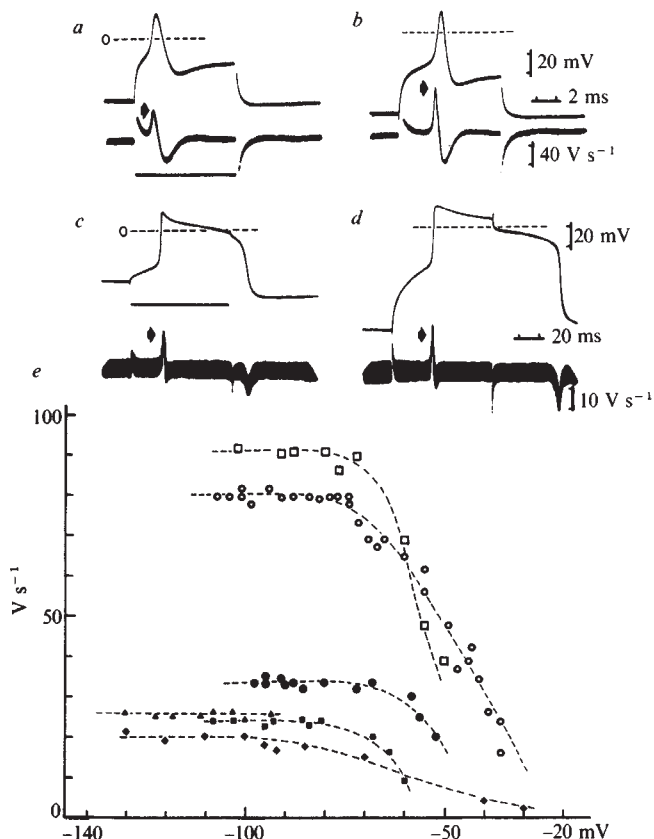


Fig. 2 Na and Ca spikes, and dependence of their MRRs on intracellular potential. *a* and *b*, Na spikes from a nerve cell after 3 days in cell culture at -62 mV (*a*) and -89 mV (*b*); the holding membrane potential was changed by d.c. current injection through the recording microelectrode. Bar below the record shows a period when a depolarising pulse current is injected intracellularly. Arrow in the lower trace shows the MRR of the spike. Extracellular solution was the tissue culture medium, which contained about 141.7 mM Na^+ ; 5.0 mM K^+ ; 1.9 mM Ca^{2+} ; 0.5 mM Mg^{2+} ; 119.5 mM Cl^- ; 25.9 mM HCO_3^- ; $0.9\text{ mM H}_2\text{PO}_4^-$; 0.5 mM SO_4^{2-} ; 5.5 mM glucose ; 1.6% protein, and a small amount of amino acids, vitamins, antibiotics and phenol red; pH of the solution was $\sim 7.4\text{--}7.6$ during the experiment, and was monitored continuously by colour of phenol red. *c* and *d*, Ca spikes at -51 mV (*c*) and -100 mV (*d*), elicited in a 5-d nerve cell. Repetition rate of the membrane stimulation was kept at less than 2 to 4 per min. External Na-free medium contained 133.0 mM Tris-HCl ; 10.0 mM CaCl_2 ; 10.0 mM TEA-Cl ; 5.5 mM KCl ; 5.5 mM glucose and a small amount of phenol red at pH 7.4 (for all Ca spikes in this figure). *e*, Dependence of MRRs of Na and Ca spikes on the holding membrane potential. Different symbols indicate spikes from different cells. Open symbols ($\circ$ and $\square$) are Na spikes from two nerve cells and filled symbols ($\bullet$, $\blacktriangle$, $\blacksquare$, $\blacklozenge$) are Ca spikes from four nerve cells, respectively. When the membrane potential was held below -80 mV , the MRRs of both the Na and Ca spikes were maximal and steady values regardless of the membrane potential.

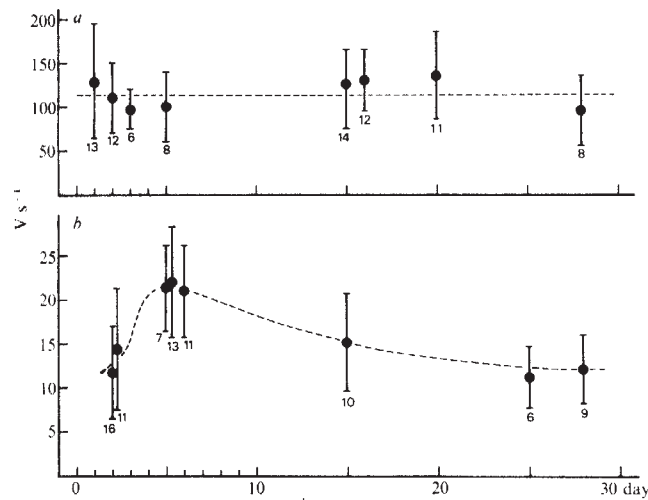


Fig. 3 Changes in the membrane capability of initiating inward Na and Ca current during cell culture. *a*, MRRs of Na spikes and *b*, MRRs of Ca spikes in volts per second. Na spikes were elicited in the cell culture medium (see Fig. 2 legend and text). Ca spikes were elicited in Na-free medium containing 63 mM Tris-HCl; 80 mM TEA-Cl; 10 mM CaCl_2 ; 5.5 mM KCl and 5.5 mM glucose at pH 7.4. Filled circles indicate mean values of the MRRs from nerve cells in dishes of the same lot, and bars are their standard deviations. Numbers of nerve cells sampled are indicated below each point.

potential (Fig. 2c), hyperpolarisation of the membrane potential by d.c. current injection removed this inactivation (Fig. 2d) and the MRR of the Ca spike reached a steady value (Fig. 2e: filled symbols). This value may be considered to represent a capability of carrying the inward Ca current of the neuronal membrane.

The MRRs of the Na and Ca spikes thus obtained were compared for cell cultures of nerve cells of various ages (Fig. 3). The MRRs of the Na spikes did not change appreciably during the 4-week period of cell culture in this study (Fig. 3a). By contrast, the MRRs of the Ca spikes exhibited a temporary increase after about 5 to 6 days in cell culture (Fig. 3b), and thereafter, decayed relatively slowly to a steady level which was close to that obtained at the beginning of the nerve cell culture. This temporary increase by a factor of 1.6, relative to the initial or the later level, is statistically significant ($P < 0.05$). It may be significant that when the MRRs of the Ca spikes are large, the nerve cells seem to be actively extending their neurites (Fig. 1c), suggesting that the increment in the MRRs may be related to neurite outgrowth.

Assuming that capacitance of unit area of the membrane²⁰ remained unchanged, the temporary increase in the MRRs of the Ca spikes can be interpreted as (1) an increase in the number of the Ca channels in the unit area of the soma membrane; or (2) a conformation change in the Ca channel molecules, which allows entry of a larger amount of Ca^{2+} . There are, however, other possibilities such as (3), an increase in the sensitivity of the K current to TEA⁺; a part of the K current which remained in the TEA-containing medium might become smaller during the particular period of the cell culture. All these possibilities reflect a definite change at least in the plasma membrane. A further possibility, (4), is an increment in the electromotive force (e.m.f.) of the Ca current; this seems improbable, because, assuming an intracellular Ca concentration ($[\text{Ca}^{2+}]_i$) ranging between 10^{-6} and 10^{-8} M in the early period of the cell culture^{27,28}, a 1.5-fold increment of the e.m.f. can be induced only by a new $[\text{Ca}^{2+}]_i$ of between 10^{-8} and 10^{-11} M, that is, 1/100 to 1/1,000 of the initial $[\text{Ca}^{2+}]_i$.

The physiological functions of Ca spikes recorded from the soma of nerve cells are not as clearly understood as those at presynaptic terminals²⁹. Hammerschlag *et al.*^{30,31} consider that

amino acid uptake and axonal transportation of proteins are intimately related to Ca entry through the soma membrane. It has also been suggested that Ca entry associated with the firing of nerve cells may be an important part of Ca utilised for axonal transportation³². In the present study, active neurite outgrowth was seen at least for several days of an early period of the cell culture (Fig. 1b, c), and simultaneously, enhancement of the MRRs of the Ca spikes was observed (Fig. 3b). During this period of the cell culture, the nerve cells must take up large amounts of nutrients and carry out extensive protein transportation in order to produce new neurites. Ca requirement associated with membrane synthesis and neurite outgrowth may be related to the possible enrichment of the Ca channels in the neuronal membrane.

The reduction of the MRRs of the Ca spikes which was observed in the later period of the cell culture (Fig. 3b) suggests that Ca requirement of the nerve cells may have been reduced for some reason (for example, reduction of neurite growth) or that a membrane part where the Ca channels were populated moved to a position remote from the soma. In the later period, however, observation of the neurites of a nerve cell became difficult because of the rapid proliferation of fibroblasts and the complex interactions of neurites originated from many nerve cells. Further study is thus required to investigate the relationship between the neurite growth and change in the Ca spikes recorded from the soma, especially that in the later period of the nerve cell culture.

Note, however, that there are many other possibilities which could explain the background of the change in the Ca spikes. Whatever the interpretation, it is interesting that ionic channels can alter their distribution or conformation relatively flexibly in the plasma membrane of the mature nerve cells.

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Neural influence on acetylcholine receptor clusters in embryonic development of skeletal muscles

THE extent to which innervation regulates the embryonic development of skeletal muscle, and the mechanisms of nerve-muscle trophic interactions during development, are little understood. Here we describe studies on the development of skeletal muscles in rat embryos in which all the motoneurons were destroyed, and experiments in which chronic application of tetrodotoxin (TTX) to individual embryos blocked all nerve and muscle electrical activity during neuromuscular junction formation. The results are presented with special emphasis on the regulation of acetylcholine (ACh) receptor distribution. It is already known that ACh receptors in adult tissues are densely clustered under nerve terminals on both muscle¹ and nerve cells². Relatively few receptors are found in extrajunctional regions, and if these are present at all they are uniformly dispersed. Studies of individually teased muscle fibres from rat embryos have shown receptor aggregates (clusters or hotspots) to be present after 16 d gestation³, but no more is known about how these clusters are induced to form or to achieve their proper location. Studies of mouse, chick and toad muscles in tissue culture have shown that receptor clusters may form on myotubes that have developed in the absence of nerve tissue⁴⁻⁶. In cultured toad muscle these clusters migrate within the muscle membrane to locate beneath nerve terminals⁵. Here, we show that receptor clusters appear on embryonic rat muscle after 15½ d gestation, with striking synchrony in timing, and an ordering of the position of clusters on different fibres within a muscle. These initial events were little changed after irreversible destruction of motoneurons at 14 d gestation except that additional receptor clusters appeared at later times. Paralysis of developing nerve and muscle with TTX did not affect the appearance and progressive increase in size of junctional clusters, but extrajunctional clusters also appeared, as in the denervated muscles. These experiments show that the first appearance of ACh receptor clusters on embryonic skeletal muscle does not depend

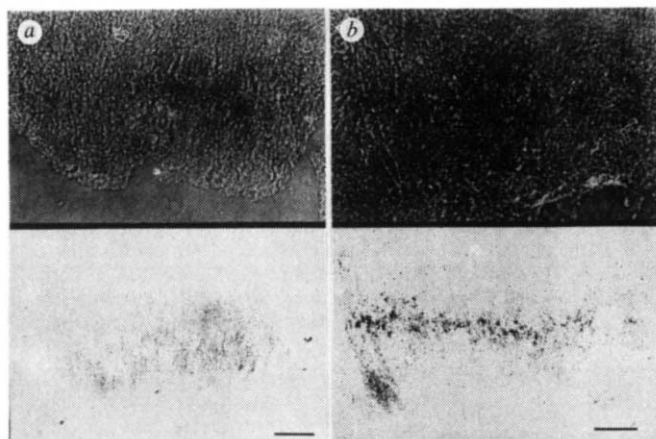


Fig. 1 Autoradiographic demonstration of ACh receptor distribution on early myotubes. *a*, Rat embryo diaphragm muscle after 15 d gestation. Top, phase contrast view of unstained section, showing narrow band of myotubes in the muscle centre, surrounded by myoblasts. Bottom, bright field view to show receptors diffusely scattered over myotubes, but not over myoblasts. No receptor clusters can be resolved at this time. *b*, Muscle after 15½ d gestation, illustrating formation of receptor clusters. Scale bars, 100 μm . Embryos were dated by taking 9 am on the morning when a copulation plug was seen as time zero. Embryos were examined between 9 and 11 am (d), or between 7 and 11 pm (½). Receptor localisation was mapped by incubating muscles with ^{125}I - α -Naja toxin, washing overnight, fixing in glutaraldehyde-paraformaldehyde, making 20- μm frozen sections, and exposing autoradiographs at 4 °C for 3 days.

on innervation, but that the final ordering of their position on muscle fibres and their later increase in size do require the presence, although not the electrical activity, of the nerve. Electrical activity of nerve and/or muscle is essential to suppress the appearance of extrajunctional receptor clusters, and for the normal progress of muscle growth. This study shows also that mammalian embryos are more accessible to experimental manipulation than may previously have been realised, so that toxins and metabolites may be chronically applied to embryonic tissues *in vivo*, as well as in tissue culture.

Motoneurons were destroyed by injection of 1 μg of β -bungarotoxin⁷ into individual embryos of 14 d gestation or older. Paralysis was not seen in embryos injected at 13 d or earlier. This toxin has been shown to destroy motor nerve terminals⁸, and to cause total loss of motoneurons following injection into chick embryos⁹. We found that a single injection of 1 μg of toxin into individual rat embryos caused a sustained loss of muscle innervation. No recovery of innervation was seen in embryos injected at 14 d and examined at 22 d of gestation. Our evidence for loss of motoneurons in rat embryos includes the absence of nerve fibres in sections of ventral roots examined under the electron microscope, and the absence of large ventral horn neurones in spinal cords of treated embryos. Toxin-treated embryos developed in a grossly normal fashion, although they were lighter in weight than their siblings, and abnormally flexed in posture.

Embryos were paralysed by inserting glass capillaries filled with 1 to 2 μl of 0.03 M TTX into their thoracic or abdominal cavities. One end of each capillary had a pore of 20 μm diameter by 100 μm length to allow slow release of the drug by diffusion. In all cases in which capillaries were fully internalised, embryos remained completely paralysed for at least 3 days, and in control experiments newborn rats were paralysed within 5 min of intra-abdominal insertion of a capillary. Survival rates of 100% were common in groups of embryos treated from 16 d gestation onwards, but implantation of capillaries into 15 d embryos was more difficult.

Clusters of ACh receptors were first seen in normal muscles at 15½ d gestation; before that receptors were diffusely distributed along the developing myotubes (Fig. 1). When first seen, junctional clusters had a mean longest dimension of $7.84 \pm 0.65 \mu\text{m}$ ($\pm$ s.e.m.). If clusters were present they were seen on the great majority of myotubes within a muscle, indicating that their time of appearance was coordinated throughout the tissue. They were not randomly positioned, but appeared as a band across the midpoint of the developing muscle.

ACh receptor clusters formed on muscle fibres made aneural by injection of β -bungarotoxin at 14 d gestation. Clusters were first seen at 16 d gestation, when they formed a broad band across the middle of the muscle. By 17 d gestation their distribution appeared more widespread than in controls although they did not extend all the way to the tendon ends of muscle fibres. In muscles treated at 14 d and examined at 20 d clusters were randomly positioned along the full length of the muscle (Fig. 2*a*), with no special accumulation in the middle of the muscle. This is in contrast to normal innervated muscle fibres which have a single cluster near the midpoint of the cell^{1,3,10}. Once present, these extrajunctional clusters retained a constant size, with mean maximum dimensions of $8.97 \pm 0.25 \mu\text{m}$ at 16 d, and $8.96 \pm 0.22 \mu\text{m}$ at 20 d gestation. If nerve terminals were destroyed at 17 d and muscles examined after 20 d gestation the endplate clusters remained, and extrajunctional clusters appeared only near the ends of the myotubes, presumably on parts of the muscle that had developed after injection of the toxin. The two types of cluster could be distinguished by their size (9.98 ± 0.26 and $6.63 \pm 0.13 \mu\text{m}$) for junctional and extrajunctional clusters, respectively, as well as by their location. Control junctional clusters in untreated littermates at 20 d were $13.28 \pm 0.29 \mu\text{m}$ in length, showing that junctional clusters in the treated muscles ceased their growth on application of toxin.

Paralysis with TTX from 16–19 d or 17–20 d gestation resulted in muscles almost indistinguishable from those treated



Fig. 2 Autoradiographic demonstration of ACh receptor distribution on embryonic rat diaphragm muscles after denervation or paralysis with TTX. *a*, Aneurial muscle from embryo after 20-d gestation injected with β -bungarotoxin at 14 d. Receptor clusters are scattered randomly from end to end of the muscle, and no endplate zone can be distinguished. *b*, Muscle from 20-d embryo following paralysis with TTX from 17 d onwards. Endplate clusters are present near the centre of the muscle, with extrajunctional clusters near the fibre ends. *c*, Muscle from 19-d embryo following paralysis with TTX from 16 d onwards. There is no clear area between endplate clusters and extrajunctional clusters. Individual silver grains cannot clearly be resolved in these low-power micrographs (scale bar, 500 μ m). *d* And *e*, receptor distribution on muscle fibres teased from 16 d–19 d TTX-treated muscle, to show that multiple clusters were present along the length of single muscle fibres. Scale bars 50 μ m. Pictures similar to those in *b–e* were obtained from muscles after β -bungarotoxin injections at 17 or 16 d, respectively.

with β -bungarotoxin at the same initial times. Paralysis during the period 16–19 d produced muscles in which junctional clusters could clearly be distinguished from extrajunctional clusters (Fig. 2*c*), but with no cluster-free regions of muscle membrane. Paralysis from 17–20 d gave rise to muscles with a clear area containing only diffusely distributed receptors between the junctional and extrajunctional clusters (Fig. 2*b*). The only distinction to be made between β -bungarotoxin-treated and TTX-treated muscles was in the size of the junctional clusters; for example 17–20 d TTX-treated muscles had junctional clusters with mean dimensions of $13.98 \pm 0.34 \mu$ m, similar to those of 20 d controls. Extrajunctional clusters were $7.46 \pm 0.14 \mu$ m in length.

Paralysis with TTX from 15–18 d produced muscles less comparable with β -bungarotoxin-treated tissues. The one control (water-filled capillary) embryo which survived from this

time was normal with respect both to gross development and to ACh receptor distribution, indicating that effects on treated embryos were due to the TTX and not to insertion of the capillary. Of three treated embryos which so far have survived, one was dwarfed and two appeared normal when examined at 18 d gestation. Muscle development was severely retarded, with myotubes only in the centre of the diaphragm muscles, surrounded by large areas of unfused myoblasts. After the TTX had been washed off, isolated muscles contracted in response to nerve stimulation. Junctional clusters of normal size ($13.70 \pm 0.33 \mu$ m) were present, but there were few extrajunctional clusters.

Diaphragm muscles contracted in response to nerve stimulation at 15-d gestation, before clusters of ACh receptors could be resolved with our techniques. Intracellular recording from 15-d intercostal muscles (M. J. Dennis and A.J.H., unpublished)

showed that nerve stimulation produced endplate potentials in every muscle fibre tested (50/50), and that miniature endplate potentials at this age were much smaller than in 16-d muscles, despite similar resting potentials (>70 mV) at both times. Nerve fibres are physically present in developing muscles before fusion of myoblasts into myotubes has begun¹¹. Thus muscles contract in response to nerve impulses at least 6 h before ACh receptor clusters can be resolved. It is our impression that clusters first appear as distinct units, rather than growing by progressive aggregation of dispersed receptors. It is interesting to note that once an area of muscle has developed without receptor clusters they cannot appear there later, so that denervation leads only to an increase in the density of dispersed receptors in that region. That this is not simply a matter of time was shown by denervating rat intercostal muscles at birth, and examining muscles in which denervation was maintained for up to 4 weeks; extra-junctional clusters appeared only near the tendon ends of the muscle fibres, in regions which had developed since the time of denervation.

Neither destruction of the innervation nor paralysis of nerves and muscles in rat embryos prevented the formation of receptor clusters, and fusion of myoblasts into myotubes was only retarded and not arrested by these procedures. We conclude that, by evoking muscle contraction, innervation speeds the expression of a developmental programme inherent in myogenic cells for elongation and growth of myotubes and for the first appearance of ACh receptor clusters. The effects of electrical stimulation or of application of TTX to spontaneously contracting monocular cultures of myotubes in tissue culture are consistent with this description⁴. A specific inductive action¹² of the nerve terminal, exerted independently of electrical activity, must be postulated to regulate the positioning of the cluster under the nerve terminal^{5,13} and for its progressive increase in size³. In the presence of a nerve terminal electrical activation of the muscle prevents the appearance of further receptor aggregates. TTX treatment over the period 15–18 d had an effect more drastic than denervation, perhaps by affecting spontaneous as well as nerve-induced muscle contractions, and so retarding muscle growth. If the appearance of extra-junctional clusters in denervated muscles is secondary to muscle growth, as seems likely (Fig. 2b), their apparently paradoxical absence from the 15–18-d treated muscles may be explained by the muscles being in a developmental stage reached by β -bungarotoxin-treated tissues at 16½ or 17 d. Further experiments are needed to clarify this point.

The only known action of TTX is to block the action potential mechanism in excitable tissues. Chronic local application to mature motor nerves causes prolonged paralysis but only partially blocks the trophic effect of the nerve on muscles^{14,15}. The residual trophic action of the paralysed nerve may depend on materials carried by axonal transport¹⁶, although this is debatable¹⁷. Electrical stimulation of adult motoneurons increases the amount of proteins carried by axonal transport¹⁸, and as application of TTX to embryos blocked action potentials in motoneurone cell bodies as well as axons this might have reduced the rate of synthesis of the hypothetical trophic substances. Thus the argument made above for electrical activation being of primary significance in nervous regulation of embryonic muscle development is the most economical, but it is not definitive.

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Composition and control of secretions from tracheal bronchial submucosal glands

TRACHEAL bronchial submucosal (TBS) glands are thought to supply a significant fraction of respiratory tract fluid (RTF)¹, which is critically important in cleaning the airways of the lung. In the cleaning process, cilia in the tracheal bronchial tree continuously sweep the RTF, with debris, out of the lung. But abnormalities in the physicochemical properties of the RTF may impair this clearance, resulting in fluid stagnation, infection and obstruction of the airways. Unfortunately, because of the small size and inaccessibility of TBS glands and the difficulty in collecting their uncontaminated secretions, little is known about their physiology. We have developed methods for collecting and analysing secretions from single TBS glands of the cat *in vitro*, and we describe here the composition and control of the fluid secreted by these glands. We have found that the composition of the secreted fluids is very similar to that of the extracellular fluid and that secretion flow rates are influenced predominantly by α -adrenergic and cholinergic agonists. In view of the well known exocrinopathy in the lethal genetic disease cystic fibrosis (CF)², we believe these findings to have implications for pathological changes in the airways in this disease.

Tracheae were excised from seven mongrel cats anaesthetised with pentobarbital (35 mg per kg). Using a modification of a technique³ for observing secretion *in vivo*, the tracheae were opened along the dorsal fold, and the epithelial surface was dried quickly with cotton sponges and a jet of freon-13 gas. The surface was coated immediately with water-saturated paraffin oil, and a segment of the trachea (about 1 cm²) was mounted in a chamber such that the serosal tissues were constantly bathed in Ringer solution which mimicked interstitial fluid (Table 1), while the epithelial surface remained covered with oil. Secretion was stimulated by adding appropriate concentrations of bethanechol (cholinergic agonist), methoxamine (α -adrenergic agonist) and isoprenaline (β -adrenergic agonist) to the bath. Under a dissecting microscope, small droplets of secretory fluid were observed to form on the tracheal epithelial surface shortly after stimulation. Timed collections of droplets secreted from three to four glands were taken up between oil blocks in constant-bore capillaries (78 μ m inner diameter), and droplet volumes were measured for rate determinations usually over a period of about 5 min. The elemental chemical composition of each sample was assayed by microdroplet X-ray analysis for Na, K, Ca, Cl, S, and P (ref. 4).

Secretory rates reached maximal values about 10 min after the application of agonists, after which the rates generally declined (Fig. 1). Maximal rates as high as 20 nl per min per gland were induced but not sustained by bethanechol or methoxamine. Maximal secretory rates with 10^{-5} M isoprenaline in the bath were generally less than 10% of the maximal rate induced by 10^{-6} M bethanechol or 10^{-5} M methoxamine (Fig. 1). Glands generally responded to stimulation for at least 5 h *in vitro*, but, probably due to variation in gland sizes, there was considerable variation in secretory rates between glands.

The composition of secretions was basically the same as that of the bath. There were no significant changes in the concentrations of any of the principal electrolytes (Na, Cl, K or Ca) as a function of the secretory rate or the nature of the applied stimulant (Table 1). Hence, if TBS glands normally secrete essentially isotonic fluids *in vivo* we conclude that, unlike most other exocrine glands, the cat TBS gland does not significantly modify the electrolyte composition of the primary fluid in the ductal region before it is excreted.

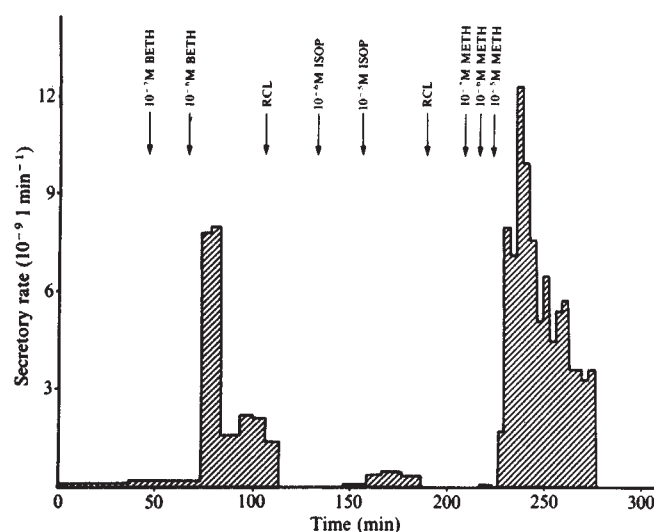


Fig. 1 A typical secretory rate response (ordinate) of a single TBS gland to increasing concentrations of bethanechol (BETH), isoprenaline (ISOP) and methoxamine (METH) in the bathing media. The time of addition of each drug at the indicated concentration is shown by the position of the inverted arrow relative to the abscissa. Rinsing was carried out by changing the bath to Ringer's solution (RCL). Concentrations, an order of magnitude higher than those shown, were examined in trials, but did not reveal noticeably higher maximal rates, even though rinsing times were increased.

However, a notable exception to this otherwise relatively constant composition is in the mean concentration of sulphur, which varied significantly depending on the nature of the stimulating agent. Methoxamine-stimulated secretions contained significantly less S (2.1 mM) than either bethanechol (3.2 mM; $P < 0.025$) or isoprenaline (4.8 mM; $P < 0.005$) stimulated secretions, but the difference between the mean concentration of S in bethanechol and isoprenaline stimulated secretions was not statistically significant ($P < 0.1$). Sulphur concentration varied widely among glands (range 1–16 mM) and was inversely correlated with secretory rate (for individual glands, correlation coefficients ranged from -0.33 to -0.82).

Both the wide variation in the S concentration and the negative correlation with secretory rate indicate that S appears in the gland secretion by a route different from that of elements in the precursor fluid. Because isotopic S is taken up by TBS gland cells⁵ and incorporated into the glycoprotein component of tracheal secretions⁶, mucus is the most probable additional source of S. If the concentration of S reflects the mucus content of the secretions, the data indicate that isoprenaline stimulates secretions with low flow rates but relatively high mucus content,

Table 1 Concentrations (mM) of the major inorganic elements in TBS gland secretions after stimulation with α - and β -adrenergic and cholinergic agonists

	Na	Cl	K	Ca	P	S
Methoxamine (α -adrenergic) $n \geq 35$	135 ± 1.7	119 ± 1.7	5.7 ± 0.18	0.6 ± 0.06	0.9 ± 0.11	2.1 ± 0.27
Bethanechol (cholinergic) $n \geq 13$	134 ± 2.1	124 ± 1.9	6.3 ± 0.28	0.7 ± 0.12	1.3 ± 0.14	3.2 ± 0.4
Isoprenaline (β -adrenergic) $n \geq 13$	141 ± 2.7	126 ± 2.5	5.7 ± 0.30	0.6 ± 0.08	1.1 ± 0.18	4.8 ± 1.09
Incubation	136	119	5.0	2.0	1.2	1.2

Except for S there was no statistically significant difference in the concentration of any element among secretions stimulated with the different agonists. Sulphur in methoxamine-stimulated secretions was significantly lower than S concentrations in the other two groups of secretions as determined by Student's *t*-test¹². Errors are standard errors of the mean; the number of samples analysed was equal to or greater than *n*.

whereas methoxamine stimulates secretions of high flow rates but relatively low mucus content. Bethanechol stimulates secretions of both high flow rates and relatively high mucus content (Table I, Fig. 1). In other exocrine glands such as the parotid, protein secretion is higher during β -adrenergic stimulation while flow rates are higher during α -adrenergic stimulation⁷. Similarly, the rates and protein content are higher with cholinomimetic stimulation in the salivary gland, but the higher protein content may be due to β -adrenergic effects of the cholinergic agonist⁸.

The observation that different stimulatory drugs evoked distinct maximal secretory rates and different S (mucus) concentrations supports the view that the physical properties of the RTF may be regulated by combined sympathetic and parasympathetic controls⁵. If the autonomic nervous system controls the relative amounts of fluid and mucus, autonomic disturbances in CF^{9,10} may produce abnormal volumes of secretions or abnormal concentrations of mucus which depress respiratory clearance mechanisms. This type of defect may explain why, although widely accepted, no defect in mucus production in this disease has been established¹¹. Also, if there is no ductal modification of the precursor fluid, the defect in CF would seem to be basically a problem of secreting appropriate volumes and mucus concentrations.

In conclusion, cat TBS glands produce a secretion which is essentially isotonic, but which varies in S and possibly mucus content as a function of the nature or degree of stimulation. The results are consistent with the possibility that malfunctions in the TBS glands in CF may alter the properties of the RTF and thereby impair airway clearance, rendering the CF lung highly susceptible to infection and obstruction.

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Clues to the site of origin of the complementary image

PATTERNS of near-parallel black-and-white lines induce an anomalous state of the visual system, in which a superimposed test field of dynamic noise (such as the 'snowstorm' on a detuned TV receiver) seems to stream in directions roughly perpendicular to the inducing contours¹. The illusory streamers form a 'complementary image' (CI) which for the stimulus of Fig. 1a comprises a family of wavy circles, as sketched by J. P. Wilson² in Fig. 1b. When two orthogonal gratings are superimposed, the streamers lie at 45° to both, as if the resulting bias obeys the rule of vector summation³. J. P. Wilson⁴ has observed rudimentary signs of directional bias and streaming even at a single black/white contour; and M. E. Wilson⁵, using only simple pairs of test spots illuminated in sequence, found that the illusory 'phi' motion seen between the spots was enhanced for directions orthogonal to the contours of a superimposed grating. When the inducing pattern is presented to one eye and the noise field to the other, however, little or no CI is observed^{1,6}. It has seemed logical¹ to attribute these phenomena to some form of adaptive cooperative activity among orientation-sensitive cells of the visual system, leading to a kind of 'simultaneous contrast' in the orientation domain, that is, a bias of the signalling system for motion and contour orientation in favour of the orientation(s) not present in the inducing stimulus.

Human observers can perceive contours outlined only by contrasts of texture or colour almost as readily as those due to differences in luminance. It would therefore help in locating the site of origin of the CI to know whether the system responsible can be directionally biased by patterns outlined only by contrasts of texture or colour. Hammond and I^{7,8} have recently found that 'simple' cells in area 17 of cats, which respond briskly to a suitably orientated moving black bar, show no response to a similar (clearly recognisable) bar of random visual texture moving over a static textured background of the same mean luminance. Although the colour sensitivity of simple cells in area 17 of primates is still controversial^{9,10}, it seems that most are also insensitive to boundaries outlined by colour contrast alone. Thus, if the CI could be induced by patterns of texture or colour contrast alone, this would suggest that it originated in a system not dependent on simple cells for its input (unless human simple cells differed in this respect from those of cat and monkey). If, however, induction of the CI required the patterns to be outlined by luminance contrast, this would point to the simple-cell system as the likely site of origin.

In the first experiment, a 60-ray pattern 12 cm in diameter, similar to Fig. 1a, was cut from white paper and placed over the screen of a detuned TV receiver generating dynamic visual noise. The paper pattern was front-lit at a luminance of 30 cd m⁻². With the mean luminance of the TV display either higher or lower than that of the paper pattern, the normal CI was clearly visible in the noise. When the mean luminance was adjusted to the same level (30 cd m⁻²), however, the CI disappeared, although the white ray pattern remained clearly perceptible by contrast with the dynamic noise between its spokes. (Some 60 observers have confirmed these findings).

An analogous experiment was then carried out using patterns outlined by colour contrast. A pair of conventional 100-W slide projectors with red and green filters (Cinemoid nos 14 and 24) were used in conjunction with a mixer cube to project on to the noise-filled TV screen the same 60-ray figure outlined at ~20 cd m⁻² in red and green. The mean luminance of the (white) noise field alone was adjustable for optimum effect. When the luminances of the interleaved red and green rays were matched the CI was absent or much reduced for all of five observers, although with the red or green rays alone (that is, one projector switched off) it was clearly visible.

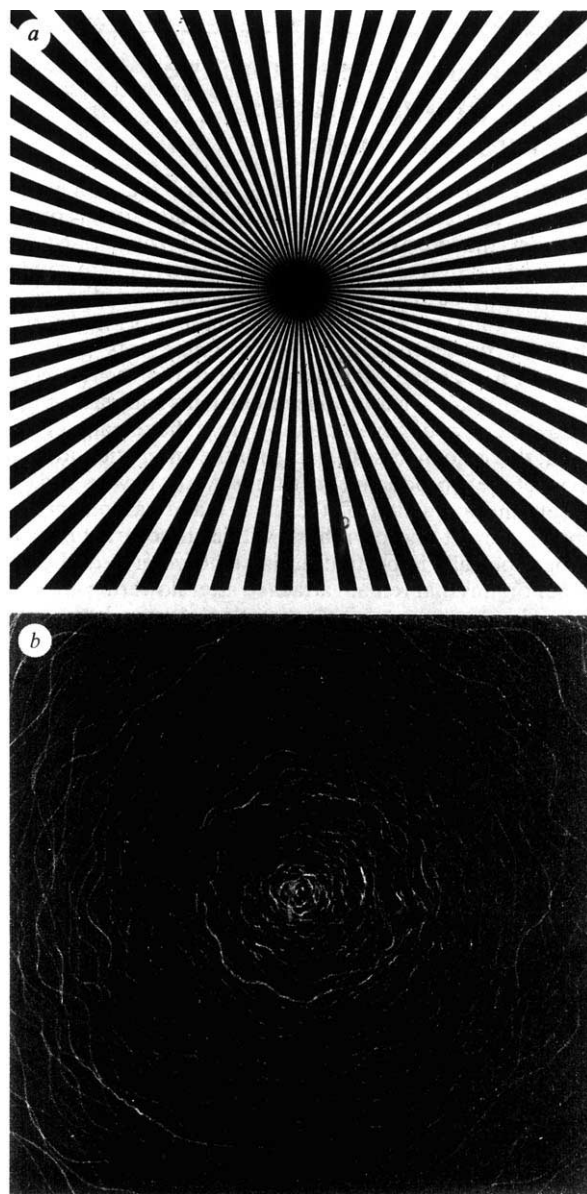


Fig. 1 a, The stimulus used for the production of a CI. b, The family of wavy circles obtained.

It therefore seems that the anomalous physiological condition responsible for the CI arises not at the level of the pattern-recognition system *per se*, but at an earlier stage of visual information processing which is specifically sensitive to contrasts of luminance, although not of texture or (perhaps) colour. The absence of significant interocular transfer suggests a location in the monocular visual sub-system. The parallel with the preferences shown by simple cells in area 17 of cats and monkeys points to these cells, or others dependent on them, as likely candidates.

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Contribution to reproductive effort by photosynthesis of flowers and fruits

REPRODUCTION is often a lethal or semi-lethal activity, and for iteroparous plants it is often possible to show that reproduction has costs that are expressed in a reduced growth rate and/or an increased death rate¹. Attempts have been made to compare life history patterns in flowering plants by measuring the fraction of a plant's annual dry matter production (or calorific value) that is allocated to reproduction (for example refs 2–4). The assumption is that the reproductive parts represent a cost in energy or materials. Clearly mineral nutrients and water must be gained by the reproductive structures from the remainder of the plant, but this is not necessarily true for the energetic and carbon economy of the reproductive structures. Many flowers and fruits are green and a fraction of the energy and carbon might be obtained by direct photosynthesis within these structures. This might be especially important during embryo growth if carpels and other organs that remain attached after flowering are green. In such cases the conventional estimation of reproductive effort (dividing seed weight by plant weight) would be incorrect and comparison of life history patterns and their evolutionary meaning would be invalid. There are reports of significant contributions of *in situ* photosynthesis in flower and seeds to their growth. Biscoe *et al.* have estimated that 47% of the carbon required for seed production in barley is provided by photosynthesis of reproductive and immediately adjacent plant structures⁵. Bazzaz and Carlson have shown that in the annual weed *Ambrosia trifida* L., net photosynthesis by reproductive structures amounts to 41 and 51%, respectively, of the carbon required for male and female inflorescences⁶. Here we report an analysis of the carbon budget of reproduction for 15 temperate deciduous trees. The budget was determined by measuring the weight, photosynthesis and dark respiration of flowers or inflorescences from bud break until seed maturity.

Inflorescences, flowers or fruits with a 3–5-cm piece of stem were excised from plants, sealed in vials containing water, and the rate of CO₂ exchange of the inflorescence was determined, first in the light (700 $\mu\text{E m}^{-2} \text{s}^{-1}$) and then in the dark

(1 $\mu\text{E m}^{-2} \text{s}^{-1}$) (ref. 7). Carbon dioxide exchange was measured at an air temperature equal to the mean daily maximum temperature (calculated from 20-yr summaries of weather data for Morrow Plot, Urbana, Illinois) for each day in which measurements were taken.

The data for photosynthetic and dark respiration rates were fitted by least squares regression to exponential, logarithmic and power univariate expressions with time as the independent variable. The expression with the largest coefficient of determination (r^2) was chosen to represent the rate of CO₂ exchange throughout reproductive development.

Weight data were fitted to a linear form of two different functions and the one with the highest r^2 was chosen to represent weight gain for that species. One of the functions used had both a lower and an upper asymptote

$$W = \frac{K}{1 + e^{c-bt}} \quad (1)$$

and the other had only an upper asymptote

$$W = K(1 - e^{-bt}) \quad (2)$$

where W = weight of flower or inflorescence in mg, K = weight at maturity in mg, e = base of natural logarithms, c = a constant of integration determining the position of the curve relative to the origin, b = the capacity for increase in units of mg/t, and t = time in days since 14 March. In fitting data to equations (1) and (2), K was estimated by trial and error to maximise r^2 . A carbon budget was constructed as described previously⁶.

There were three general types of weight gain curve for reproductive structures (Fig. 1). A continuous form of equation (1) represented the best expression for growth in 11 species (Table 1) and is exemplified by *Platanus occidentalis*. Equation (2) was the best expression for reproductive growth for two species represented by *Acer saccharum* (Fig. 1b). The third type of reproductive growth is the split or two-phase form of equation (1) as found for *Liriodendron tulipifera* and *Magnolia stellata* (Fig. 1a). Large showy petals were formed during the first part of reproductive development but fell off by about day 65, causing a sharp decrease in weight. Other species also lost some floral parts during development but that did not alter the smooth character of the growth curve.

The pattern of development described by equations (1) and (2) may reflect important differences in the metabolic cost of

Table 1 Floral sexuality, weight gain, photosynthesis and respiration, and percentage contribution to reproductive effort of ♀ flowers and seeds of selected species

	Floral sexuality	Overdry weight at maturity (mg)	Totals GP (mg)*	Total daytime respiration (mg)*	Total night-time respiration (mg)*	Total respiration 3+4 (mg)*	Net photosynthesis 2-5 (mg)*	% Gain or loss (2-5)/1 (100) †	% Contribution GP to total carbon balance 2/(1+5) ‡	Coefficient of determination (r^2)	Type 1 curve	Type 2 curve
<i>Acer platanoides</i>	H	254	312	156	74	230	82	32.3	64.5	0.894		0.980
<i>Tilia platyphyllos fastigiata</i>	H	1,868	794	283	124	407	387	20.7	34.9	0.902		0.721
<i>Acer rubrum</i>	H	28	15	13	6.8	20	-4.8	-17.1	32.9	0.946		0.922
<i>Acer saccharum</i>	H	210	132	141	47	188	-56	-26.7	33.2	0.860		0.960
<i>Magnolia stellata</i>	H	823	340	247	119	366	-26	-3.2	28.4	0.971		0.835
<i>Cercis canadensis</i>	H	208	96	93	44	137	-41	-19.7	27.9	0.961		0.958
<i>Liquidambar styraciflua</i>	M	2,375	955	1,103	510	1,613	-658	-27.7	23.9	0.999		0.945
<i>Betula pendula</i>	M	565	192	221	101	322	-130	-23.0	21.7	0.910		0.972
<i>Celtis occidentalis</i>	H	342	118	139	65	204	-86	-25.1	21.5	0.908		0.994
<i>Prunus serotina</i>	H	131	38	46	21	67	-29	-22.1	19.2	0.959		0.937
<i>Liriodendron tulipifera</i>	H	2,900	1,032	1,970	914	2,884	-1,852	-63.9	11.5§		§	0.975
<i>Ulmus americana</i>	H	7.5	0.83	0.86	0.50	1.4	-0.53	-7.1	9.3	0.999		0.989
<i>Carya ovata</i>	M	8,135	944	2,367	1,068	3,435	-2,491	-30.6	8.2	0.995		0.848
<i>Platanus occidentalis</i>	M	10,158	1,072	3,540	1,699	5,239	-4,167	-41.0	7.0	0.979		0.903
<i>Quercus macrocarpa</i>	M	3,042	104	1,008	512	1,520	-1,416	-46.5	2.3	0.966		0.685

* H, hermaphrodite; M, monoecious; GP, gross photosynthesis units of C₆H₁₀O₅.

† Gain or loss due to CO₂ exchange of propagule expressed as a percentage of propagule weight.

‡ Gross photosynthesis of propagule expressed as a percent of the total amount of carbon for production of a mature propagule (2/1+5 × 100).

§ Contribution based on two parts of weight gain curves in Fig. 3, r^2 values were 0.931 for the first part and 0.993 for the second.

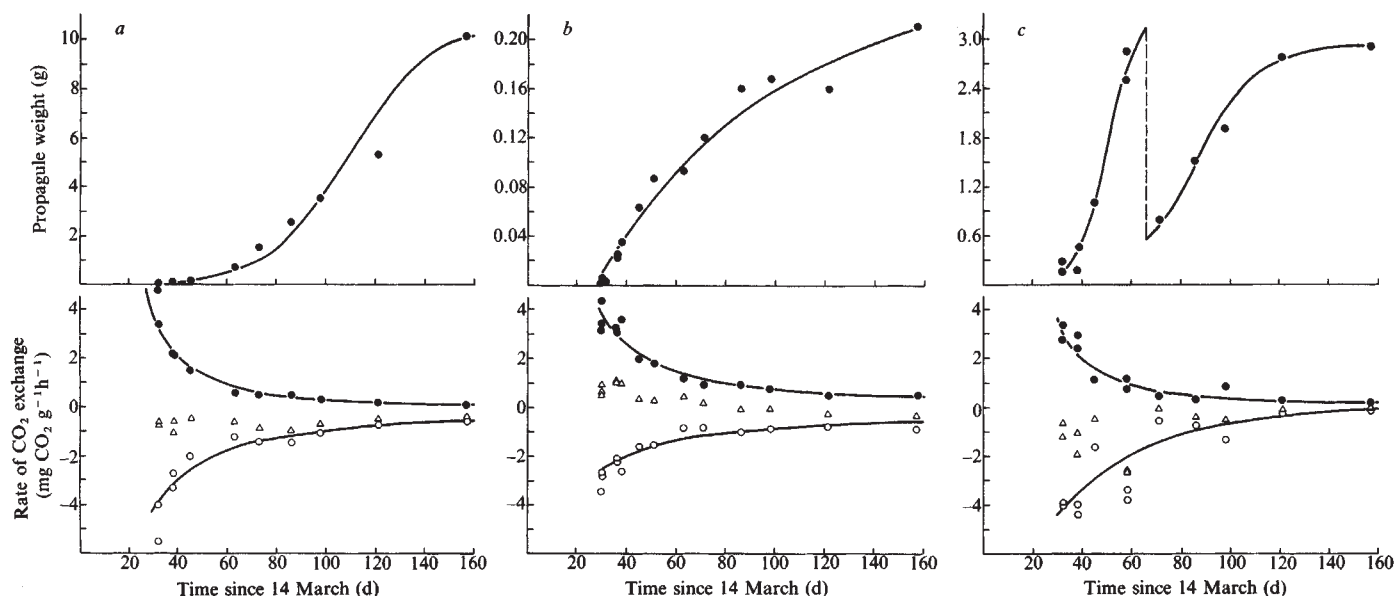


Fig. 1 Increase in growth and changes in the rates of gross photosynthesis (●), dark respiration (○) and net photosynthesis (△) in flowers and fruits of *Platanus occidentalis* (a), *Acer saccharum* (b) and *Liriodendron tulipifera* (c).

reproduction. Systems that are well described by equation (1) apparently lack an initial mass transfer of carbohydrate from the plant into the developing reproductive structure but those described by equation (2) seem to have such a transfer.

The amount of carbon supplied from *in situ* photosynthesis to the total carbon required for production of mature seed varied from 64.5% for *Acer platanoides* to 2.3% for *Quercus macrocarpa*.

The results suggest that flowers and fruits should not be regarded simply as a cost to the carbon budget of the rest of the plant. Clearly in some cases green flowers may be sufficiently active photosynthetically to pay a major fraction of the energetic and carbon cost of their own structure and that of the ripened seed. Thus it is misleading to express reproductive yield as if it is the product of leaf photosynthesis. One implication of this finding is that the energetic costs to an animal-pollinated plant with showy non-green flowers may be two-fold—both the carbon cost of making the structure and the costs of sacrificing its potential photosynthetic ability. The sharp break in the floral growth curves of the *Liriodendron*-type emphasises this point. Furthermore, in the improvement of crop plants, we should perhaps consider the extent to which the photosynthetic activity of floral structures might be improved.

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Is compensatory growth a complicating factor in mouse teratology?

METHODS developed in an attempt to optimise the chances of identifying a teratogen generally involve the administration of the compound to a pregnant female in a number of ways, followed by autopsy about 24 h before parturition. This system maximises the period during which abnormalities may develop and circumvents loss by cannibalisation of dead or deformed newborn animals by their mother. The methodology is simple and efficient but provides no information about the immediate response of the embryo to the drug and any capability it may have for regulation or recovery from damage. Thus developmental disturbances could pass unnoticed. However, the damage, for example behavioural or reproductive abnormality, may manifest itself postnatally, and this has recently aroused some concern^{1,2}. We report here on the mode of action of a known teratogen, mitomycin C (MMC) on mouse embryos. In rodents this antibiotic is teratogenic at high doses. In rats the LD₅₀ and teratogenic dose seem to coincide, but for mice these figures may differ considerably^{3–5}. Abnormalities reported include exencephaly, spina bifida⁶, skeletal and other non-nervous system defects⁷. The frequency of abnormality and dose response is strain dependent in mice. We know of no reports on the immediate response of mouse embryos to MMC and of only one on the postnatal development of mice exposed to the drug *in utero* in mid-gestation⁸. Our results indicate that, during early organogenesis stages, the mouse embryo has a remarkable capacity for recovery from severe damage and developmental retardation induced during primitive-streak stages, when drugs were thought more likely to be lethal than teratogenic.

Random bred Q strain mice were used. Pregnant females received a single injection of MMC intraperitoneally 6½ or 7 d postcoitum (p.c.). Two doses were used, 100 and 150 µg per mouse (~4 and 6 mg per kg). There were no noticeable differences in response to the two doses, so data have been pooled. In this strain the primitive streak begins to form between 6½ and 6¾ d p.c. Initially autopsies were done at 7½ and 8½ d p.c.

Table 1 Cell numbers and mitotic activity in control and MMC-treated 7½-d embryos

<i>n</i>		Control 6	MMC 5
No. of cells	Ectoderm	7,618 ± 443	978 ± 157
	Mesoderm	3,743 ± 256	374 ± 53
	Endoderm	1,019 ± 57	374 ± 33
M/A index	Ectoderm	3.9 ± 0.2	12.5 ± 1.5
	Mesoderm	1.9 ± 0.2	5.2 ± 0.7
	Endoderm	4.5 ± 0.6	6.1 ± 0.7

Camera lucida drawings were made of transverse sections and tissue volumes computed from planimeter measurements of tissue areas. Cell numbers were then calculated from a knowledge of cell volume¹³.

but were subsequently extended to various other time points, including postnatal development.

At 7½ d MMC embryos were morphologically normal but very small (Fig. 1a, b). On the other hand, the extra-embryonic regions of the egg cylinder are of normal size, but in some the mesodermal layer of the exo-coelom is incomplete. Serial reconstructions of embryos show that in MMC embryos cell numbers are reduced to about 14% of normal but that mitotic activity is much higher (Table 1). In a study of the time course of development following MMC injection at 7 d p.c. (zero time) dead cells (pycnotic granules) are observed at 1½ h and are most abundant at 6 h. Mitotic activity falls to its lowest at 6 h. The high mitotic activity in 7½-d MMC embryos seems therefore to reflect accelerated, compensatory growth in the embryo.

At 8½ d MMC embryos are still small and are now morphologically retarded (Fig. 1c, d). Headfolds are usually very small, or absent, the anterior end of the embryo usually being at the neural plate stage, that is, about 12 h retarded. In some embryos the posterior end seems more advanced, with a well developed allantois and occasionally with an early hind gut and one or two somites. The mitotic index is high throughout the embryo. Control embryos at this stage have raised head folds, neuromeres, up to seven pairs of somites, a beating heart, and fore and hind gut, and chorio-allantoic fusion is complete.

We initially thought that MMC embryos would die, but at 10½ d, although still small, they were almost normal. Head development showed no outward sign of retardation, but somite numbers were low (29 against 35 pairs in controls) and hind limb bud development was late in many. The course of development of MMC embryos is shown in Table 2. By 13½ d the only evidence for retardation in MMC embryos is that the mean fetal weight is significantly low (126 ± 3.0 mg, 5 litters, 42 embryos in MMC mice against 143 ± 3.4 mg, 4 litters, 42 embryos in controls, $t = 3.1$ $P < 0.0025$). Abnormalities were found in one litter. Among the 10 embryos there were four cases of microphthalmia and one cleft face. There were no abnormalities in controls.

Litter sizes were a little lower in MMC-treated females, all embryo mortality occurring before 13½ d of gestation. Seventeen control litters averaged 10.9 implants and 3.2% mortality, whereas in 72 MMC litters these figures were 10.7 and 10.0%.

At birth MMC newborn animals were not noticeably small but were not weighed: 6% were stillborn. In 14 litters (mean size 8.0) microphthalmia was seen in 5, in 26% of all 14-d-old mice. No other abnormalities were seen. Growth was often slow and mortality was extremely high (25%, 53% and 64% being dead at 7 d, 14 d and 21 d, respectively). Of 14-d survivors, 50% were runted. Two entire 14-d litters had the appearance of 8-d-old mice, none had its eyes open. Only 30% (29 mice) were successfully weaned.

All runts and many of their larger littermates showed motor defects. Gait was slow and stumbling and many trembled violently. Most were reluctant to move and some were easily knocked over and took 5–10 s to get back on their feet. One extreme case would lie on its side for 30 s or more before moving.

Most runts and those with the worst motor defects died before weaning. Wilson razor blade slices were made of four severely affected 14-d mice but no internal abnormalities were found, all organs being present, correctly located, of normal proportions and with no noticeably enlarged cavities. Survivors generally seemed normal at weaning.

Fertility is low. All 29 survivors were test-mated to normal Q mice of proven fertility. All 18 males mated but 8 gave sterile plugs. Histological examination revealed small testes deficient in germ cells, many tubules containing only Sertoli cells. Of the 11 females 2 were sterile; both had small ovaries with about half as many follicles as controls, and one also developed motor defects after weaning. Histological examination of MMC embryos between 7½ and 11½ d in which alkaline phosphatase-positive primordial germ cells (PGC) were counted indicated severe depletion of PGCs in all MMC embryos up to 9½ d. Subsequently about half the embryos show good recovery of germ cell numbers. Migration of PGCs was retarded, even if allowance was made for the general developmental retardation.

Several other studies have used doses of MMC similar to or higher than those used here but none has started as early in pregnancy^{9–11}. They cover the period 7½–15 d p.c.; all report high levels of induced abnormalities, up to 90% of females presenting abnormal fetuses and 64% of fetuses being affected. These figures agree well with the frequency of severe developmental retardation seen in our 8½-d embryos (100% of females present, up to 75% of fetuses affected), but do not correspond at all with the level of abnormality seen at late gestation. As all our experiments commence in pre-organogenesis stages this is perhaps not surprising. What our study does show is that the mouse embryo can withstand a major disturbance in its early development and recover to such an extent that structural abnormalities rarely emerge.

The frequency and nature of abnormality found in our 13½-d embryos gives little suggestion of the profound effects on post-

Table 2 The course of development of embryos exposed to MMC at 6½ or 7 d postcoitum

Age at autopsy (d p.c.)	Age at injection	No. of litters	No. of embryos		% of embryos showing:			Limb-bud	
			Live	Dead	Headfold		Somites	Fore	Hind
					Absent*	Normal			
8½	—	6	56	2	0	100	91	0	0
	6½	6	46	5	44	56	56	0	0
	7	10	97	9	75	25	39	0	0
9½	—	5	58	3	0	100	97	73	0
	6½	5	44	2	0	100	86	43	0
	7	12	118	8	4	96	80	6	0
10½	—	2	23	1	0	100	100	100	100
	6½	6	56	17	0	100	95	85	80
	7	4	42	4	0	100	100	92	55

* This figure includes embryos in which the neural plate has just started to crease, but raised neural folds are not observable.

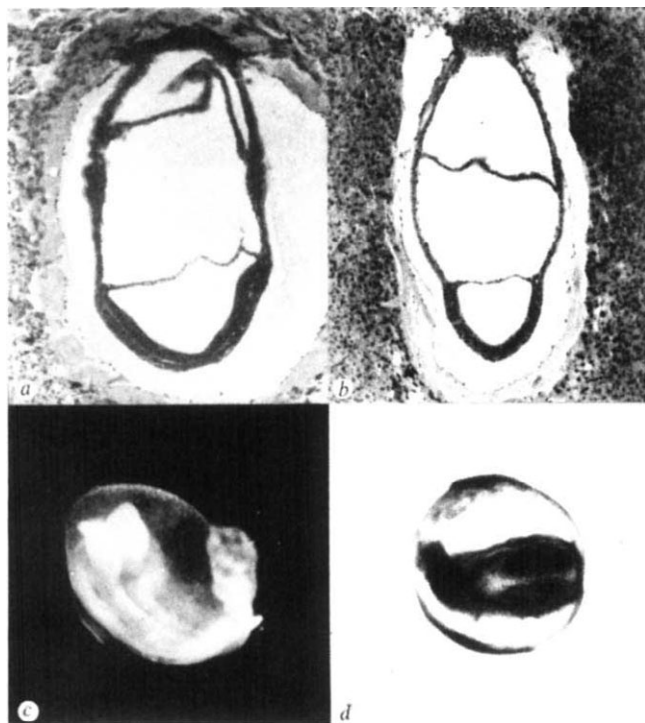


Fig. 1 *a*, Sagittal section through normal 7½-d egg cylinder. *b*, As *a* for MMC-treated embryo. *c*, Normal 8½-d embryo, dark ground illumination. *d*, MMC-treated 8½-d embryo, bright field illumination. No headfold or somite development has occurred.

natal development and fertility, of MMC applied during pre-organogenesis stages. However, MMC-induced reduction in fertility has been reported to follow treatment given in mid-gestation⁸.

We have shown that the mouse embryo can be reduced to around 10% of its normal size at a time when it is about to begin organogenesis, but is nearly normal again before that phase of development is complete. Much of this regulation, especially morphogenesis, is accomplished within 48 h of the damage being inflicted. In particular, the formation of the brain and head seems to be achieved in about half the time it normally takes. During this period of accelerated development the embryo may be more susceptible to damage which would normally be trivial. It may explain, for instance, why some teratogens have their severest effect when administered as a series of small doses rather than as a single large dose^{5,12}, a possibility not considered by Wilson in a discussion of the complications introduced by repeated dosing⁵.

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Hybrid sterility in meadowlarks

THE eastern meadowlark (*Sturnella magna*) and western meadowlark (*S. neglecta*) are sibling species of songbirds in secondary contact throughout a narrow zone of sympatry in central North America^{1–3}. Field studies suggest that specific differences in vocalisations are the principal isolating mechanism and that hybridisation is limited in extent and possibly restricted to regions of recent contact^{4–7}. The incidence of hybridisation is difficult to determine, however, due to their similar appearance, and there is uncertainty as to the recognition of some hybrids of known parentage⁷. Multivariate analysis alone offers no additional resolution to the problem⁸. A programme to breed and hybridise meadowlarks in captivity is one approach to determining the viability and fertility of hybrids. The difficulties inherent in the technique and the time required to amass adequate samples have precluded its use among avian systematists, with the exception of those working on some game species^{9,10}. However, I now report that between 1966 and 1978 I was successful in inducing 25 captive meadowlarks to pair and produce 44 clutches of 158 eggs. Mixed matings among non-hybrids resulted in 90% fertility, not significantly different from the 87% fertility among eggs from pure matings, whereas the fertility of eggs from pairing of hybrids was only 10%. All eggs resulting from pairing the one surviving backcross hybrid were infertile.

Indicative of the infertility of my hybrid meadowlarks is the performance of three siblings produced in my aviary in 1975, from a mixed pairing of a male *S. magna* and a female *S. neglecta*. The three hybrids, two males and a female, did not breed at 1 yr of age, although paired with experienced mates. In 1977 the female hybrid was paired with a male *S. neglecta* that had bred successfully the previous year. She built a typical meadowlark nest, laid a clutch of four eggs, and incubated with normal attentiveness. On the eighth day of incubation I determined that all eggs were infertile. The male *S. neglecta* demonstrated his continued fertility by breeding successfully the following year. One of the male hybrids was paired in 1977 with a female *S. neglecta* that had bred successfully 2 yr earlier. She laid a clutch of four eggs determined to be infertile after 6 days of normal incubation. Also in 1977, the second male hybrid was paired with a 2-yr-old female *S. magna*, who laid a clutch of three eggs that proved to be infertile after 10 days of normal incubation. This female laid fertile eggs the following year in a mixed pairing with a male *S. neglecta*.

My most extensive and convincing data were accumulated on a male hybrid produced from a mixed pairing of a male *S. neglecta* and a female *S. magna* in 1966 (the first meadowlark ever produced in an aviary and raised to maturity). At 1 yr of age he was backcrossed with his mother, who laid a clutch of four eggs that proved to be infertile after 11 days of normal incubation. At 2 yr, he was backcrossed again with his mother, who laid three clutches and a total of seven infertile eggs. During that same season he was paired with two different hybrid females. These crosses of F₁'s resulted in three clutches and a total of 12 infertile eggs. In 1971, at 5 yr of age, he was paired with a female *S. neglecta*, a proven breeder who laid fertile eggs in a mixed pairing that same year and continued to produce fertile eggs over the two ensuing years. This backcross resulted in a clutch of four infertile eggs incubated normally for at least 8 days.

The only fertile eggs from a pairing of a hybrid were laid by a female hybrid produced in the wild near Poughkeepsie, New York, from a mixed pairing between a male *S. neglecta* and a female *S. magna*⁷. This female hybrid was first successfully paired in 1968, when pairings with a male hybrid produced two clutches and a total of eight infertile eggs. In 1971, at 9 yr of age, she produced two fertile clutches: one of four eggs in a pairing with a male *S. neglecta*, and one of three eggs in a pairing with a male *S. magna*. However, in that same year she laid four infertile

eggs in a pairing with another male *S. neglecta* who had already bred successfully earlier in the season, and she laid a clutch of eggs in a second pairing with the male *S. magna* cited above, but the three eggs in this subsequent attempt proved to be infertile. A sibling female hybrid from the mixed pairing near Poughkeepsie was paired with one of my aviary-produced hybrids in 1968 and produced four infertile eggs. I was unable to establish breeding pairs with the other hybrids from Poughkeepsie.

Two of the backcross hybrids by the female hybrid cited above were hand-reared, but only one of these (a male, from W × F₁WE) was subsequently involved in a successful pairing. When 2 yr of age this backcross hybrid male was paired first with a female *S. neglecta*, resulting in five infertile eggs, and later with a female *S. magna*, resulting in three infertile eggs. The female *S. neglecta* had bred successfully in my aviaries during each of the previous two years; the female *S. magna* produced fertile eggs before and after this unsuccessful pairing with the backcross hybrid.

The ease with which hybrids were produced from mixed pairings of captive birds, given no choice of mates, tells us little about the incidence of hybridisation of meadowlarks in the wild, where behavioural and ecological isolating mechanisms effectively limit mixed pairings throughout most of the zone of

sympatry. The sterility of the captive hybrids is significant, however, and suggests that when infrequent hybridisation does occur in natural populations, the hybrids are at a disadvantage and are selected against, a conclusion that had been suggested earlier by Rohwer's multivariate analysis of specimens from a region of sympatry⁸. The causal factors for the sterility are as yet unknown, but apply to hybrids of both sexes and to hybrids resulting from both categories of mixed pairings. Efforts to find abnormalities in the chromosomes of the hybrids have thus far been unsuccessful. The reduction in fertility is not accompanied by an obvious reduction in viability, for the captive hybrids appeared to be as healthy and vigorous as other captives.

I thank the following for assistance in maintaining the captive birds: Mrs Vicky Lanyon, the late Edward Szalay (who was superintendent of the Kalbfleisch Field Research Station) and James Mansky (current superintendent of the station).

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Table 1 Fertility of eggs produced by captive eastern (E) and western (W) meadowlarks

Category of pairing	No. of fertile/infertile eggs in complete clutches	Total no. of fertile/infertile eggs	% Fertility
Pure pairings			
W	4/0, 3/0, 3/0, 3/0, 2/0, 2/2	17/2	0.89
E	3/1	3/1	0.75
All pure pairings		20/3	0.87
Mixed pairings (male × female)			
E × W	5/0, 5/0, 4/0, 4/0, 4/0, 4/0, 3/0, 2/0, 2/0	33/0	1.00
W × E	4/0, 4/0, 4/0, 4/1, 3/0, 3/1, 0/4*	22/6	0.79
All mixed pairings		55/6	0.90
Pairings of hybrids (male × female)			
F ₁ WE × E	0/4, 0/3, 0/2, 0/2	0/11	0
F ₁ WE × W	0/4	0/4	0
F ₁ EW × E	0/3	0/3	0
F ₁ EW × W	0/4	0/4	0
W × F ₁ WE	0/4, 4/0†	4/4	0.50
W × F ₁ EW	0/4, 0/4, 0/3	0/11	0
E × F ₁ WE	0/3, 3/0†	3/3	0.50
E × F ₁ EW	0/4, 0/3	0/7	0
F ₁ WE × F ₁ WE	0/4, 0/4, 0/4	0/12	0
Backcross hybrid × W	0/5	0/5	0
Backcross hybrid × E	0/3	0/3	0
All pairings of hybrids		7/67	0.10

Earlier attempts to breed meadowlarks failed, presumably because the flight pens were too small and offered an insufficient area of natural sod for nesting substrate⁷. Ultimately I resorted to aviaries located in a grassland habitat at the American Museum of Natural History's Kalbfleisch Field Research Station at Huntington, New York, that measured 12.2 × 4.9 × 2.7 m, thus providing 60 m² of sod per breeding pair and ample unobstructed space to permit the aerial components of courtship. The sources of the breeding stock were as follows: seven *S. magna* captured in New York and New Jersey (three as adults and four as nestlings); five *S. neglecta* captured as adults in California; four *S. neglecta* produced in my aviaries from pairings of the California birds; one *S. neglecta* trapped as a breeding adult near Poughkeepsie, New York⁷; six hybrids produced in captivity from the foregoing stock, and two hybrids produced naturally near Poughkeepsie, New York⁷. Mixed pairings were as readily achieved in the flight pens as were pure pairings. No choice experiments were conducted. Whenever possible hybrids were paired with mates known to have bred successfully to maximise the significance of the performance of the hybrids. Fertility of eggs was established by candling after a minimum of four days of incubation, when the embryos and air sacs are clearly visible.

* This clutch involved a male in at least its tenth year of age (1978) that bred successfully between 1970 and 1975; he was not paired with hybrids after 1975.

† These eggs were laid by the same female F₁ hybrid and were the only fertile eggs produced by a hybrid; four other clutches (15 eggs) produced by this hybrid were infertile.

Errata

In the letter 'Common regulatory mechanism of expression and conjugative ability of a tetracycline resistance plasmid in *Bacteroides fragilis*' by Privitera *et al.*, *Nature* **278**, 657, in paragraph 2 line 11 the molecular weights of the plasmids should read 27 and 28 × 10⁶ and in line 15, 17 × 10⁶. Similarly, in paragraph 11, line 3 the molecular weight should read 17 × 10⁶.

In the letter by H. J. Chapman, *Nature* **277**, 642, the word 'age' was omitted from the title which should read '2,390 Myr Rb-Sr whole rock age for the Scourie dykes of north-west Scotland'.

Corrigenda

In the letter 'Sulphonation of cholinergic receptor disulphide bond increases response to ACh' by A. Steinaker, *Nature* **278**, 358, lines 9–10 and the equation should read: The active agent is the sulphite ion, the reaction being



In the review article 'Facts and hypotheses of molecular chemical tunnelling' by V. I. Goldanskii, *Nature* **279**, 109, on page 113 in the left-hand column line 45, for 'Sagan^{73,76}', read 'Sagan⁷³ and Sagan and Khare⁷⁶'. In line 58 for 'Sagan considers' read 'Sagan and Khare consider' and in line 71 for 'According to Sagan^{73,76}, read 'According to Sagan⁷³ and Sagan and Khare⁷⁶'.

reviews

Arena of technological policy

Dorothy Nelkin

Directing Technology. Edited by R. Johnston and P. Gummert. Pp. 271. (Croom Helm: London, 1979.) £10.95.

BELIEF in the efficacy of technological progress is increasingly tempered by awareness of its ironies. Technological 'improvements' may cause disastrous environmental problems: drugs to stimulate the growth of beef cattle may cause cancer; 'efficient' industrial processes may threaten the health of workers; beneficial biomedical research may be done at the expense of human subjects; and a new airport may turn a neighbourhood into a sonic garbage dump. Even efforts to control technology may impose inequities, as new standards and regulations pit the quality of life against economic growth and the expectation of progress and prosperity. Indeed, the public policy discourse on directing technology has changed remarkably in the past ten years. How to promote and utilise technology for socially useful ends remains an important but no longer central issue; attention has shifted to a greater emphasis on ways of assessing and controlling potentially undesirable impacts.

Directing Technology is a book of thirteen essays edited by Ron Johnston and Philip Gummert, both from the Department of Liberal Studies in Science at the University of Manchester. The essays address the two thrusts of government involvement in the direction of technology: promotion through the encouragement of research and development, and control through regulation. Government intervention has increased in recent years, fostered by the growth and the scale of public investment in technology, by the importance of technology in international economics and politics, and by the increase in public criticism and concern. Various patterns of government promotion of technology through encouraging research and development are explored in six case studies in the

first part of the volume. They include cases in Western Europe (Keith Pavitt), Canada (Ian Chapman) and Great Britain (Philip Gummert). Examples are drawn from specific industries: nuclear power (Roger Williams), aviation (John Hartland and Michael Gibbon), and motor vehicles (Peter Stubbs).

The second half of the book focuses on mechanisms of control. The focus is primarily on the problem of safety, with chapters on the regulation of the automobile industry (Judith Reppy), on the environmental impacts of technology (Dave Ewa and Harry Rothman), on pesticides (Brendan Gillespie), and on recombinant DNA (Edward Yoxen). Various mechanisms of control are discussed in this section: technology assessment (Michael Gibbons) and the role of parliaments (John Hartland).

The book concludes with a most interesting article by Geoffrey Price, which—perhaps deliberately—serves as a critique of the entire volume. Price observes that policies for promotion and control of technology express social goals and priorities:

The differing approaches to the control of technology are themselves an expression of the basic assumptions made within different political traditions about the coordination of general social goals with the detailed activities needed to attain them. In short, approaches to the regulation of technology are themselves particular instances of the political assumptions which articulate a given society—whether they be those of centralised planning, Burkean conservative liberalism, or radical communitarianism.

Price goes on to argue, as I would, for more overt recognition of the political context of decision-making concerning the direction of technology. The political assumptions underlying technological decisions are often unstated, because it is convenient to maintain an image of neutral technology. But clearly decisions about promotion and control have significant social and political consequences. They may effect employment, the distribution of income,

or occupational health and safety. The collaboration between industry and government discussed throughout this volume may have serious political implications for the ability to control technology, as the functions of promotion and control necessarily come into conflict. Can we talk these days of directing technology without discussing the problems of scientific research as well? Can we talk about the government's role in the promotion of technology without dealing with the contradiction between its efforts to promote as well as to control?

Although this book is a very useful and timely collection of articles, I wish the editors had presented a detailed and critical analysis of such politically controversial problems. Several of the authors touch on political issues; the declining capacity of parliament to influence technology, or the secrecy of government institutions in dealing with the problem of pesticides. But, for the most part, the articles are relatively bloodless and bland. It is as if decisions in this highly political arena of technology policy—political in the sense of its consequences for the distribution of costs and benefits—occur in an ideological vacuum.

One way to approach these political assumptions would be to develop the comparisons implicit in the essays. Different countries approach common technological problems in different ways depending on political expectations and cultural styles. Comparative analysis can reveal assumptions underlying decision-making processes and they can suggest a wider range of policy options. A lack of comparative analysis more generally marks the field of science and technology policy. This book, with its case-studies from different countries, at least provides us with the opportunity. □

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Speculations on micromolecular evolution

The Genetic Mechanism and the Origin of Life. By L. S. Dillon. Pp. 563. (Plenum: New York and London, 1978.)

I FOUND this to be a strange book. The list of almost 3,100 references occupies 127 of its 562 pages. This does not include the space in the text occupied by citations; I counted 23 on one page. Obviously, only perfunctory mention can be made of most articles to which the citations refer. An analogy to describe the organisation of the first five chapters is that it seemed as if the author had been out in the fields with a butterfly net, industriously scooping up insects, which he sets before us in a display bewildering in its variety. His collection of topics includes everything from the origin of life, to the complete two-dimensional structure of MS-2 RNA, and the amino acid sequences of six calf-thymus and sea-urchin histones.

After this *tour-de-force*, the author proceeds to invent new theories from the stepwise evolutionary formulation of larger amino acids from smaller ones, such as alanine from glycine, and valine from aspartic acid. He says, for example, "In the author's laboratory, continuous bubbling of CO₂ through a 0.1 [sic] aqueous solution of alanine at ambient and elevated temperatures for a week failed to produce any detectable aspartic acid (Dillon, unpublished)", but he doesn't give up; he next says, "Hence, the addition can probably be made only through enzymatic mediation". He does not mention that aspartic acid is formed biologically by transamination, but not from alanine. Grandiose but unrealistic schemes are also set forth for "chemical evolution" of lysine, isoleucine and other amino acids from aspartic acid.

In chapter 7, Dillon proceeds to take sequences of tRNAs apart into small pieces and put them together again. He says that the absence of unmodified adenosine from the first site in anticodons is included in constraints which "to the author's knowledge . . . have not been previously noted in the literature". Actually, this is a well-known characteristic and has a familiar explanation, not mentioned by Dillon. Adenosine in the first position of anticodons is deaminated to inosine, (*Biochim. Biophys. Acta*, 213, 352, 1970), which wobble-pairs with three different bases. The incorrect statement is made on p296 that "the triplet -CAA is consistently present at the 3'-terminus of the molecule" of tRNA.

The next chapter, on "tRNA evolution", attempts at great lengths to construct a phylogeny by comparing base sequences of various arms of tRNA molecules. Such a procedure is at variance with results obtained by aligning and comparing the complete sequences of tRNA molecules (*J. Molec. Biol.* 78, 91; 1973), a method which shows that sequence changes have been so extensive as to obscure any phylogenetic relationship of nearly all tRNAs except for a few closely related pairs.

In chapter 9, Dillon goes butterfly-collecting for odds and ends among the structures of viruses. At one point he briefly notes, but does not describe, the "blocked complex 5'-terminal struc-

ture" in reovirus and polyhedrosis virus RNA, discovered by Miura *et al.* (p402). This is, of course, the "cap" of eukaryotic mRNA, an appendage which Dillon (p131) strangely ignored in describing the structure of eukaryotic mRNA. The book concludes (chapter 10) with highly imaginative (or imaginary) and grandiose scenarios for the origin of life, including schemes for the stepwise evolution of small viruses into large ones. The voluminous bibliography, following chapter 10, is probably the most useful feature of the book.

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Protein phosphorylation

Cyclic Nucleotides, Phosphorylated Proteins, and Neuronal Function. Distinguished Lecture Series of the Society of General Physiologists. Vol. 1. By P. Greengard. Pp. 124. (Raven: New York, 1978.) \$15.60.

IN this slim volume, Professor Greengard expounds his theory that protein phosphorylation is the final common pathway that mediates the biological effects of a wide variety of regulatory agents in addition to those that are known to act through cyclic AMP. It is really an extension of the 'second messenger' theory of cyclic AMP function, in that protein phosphorylation is depicted as the immediate and physiologically important result of increased cyclic AMP levels. However, Professor Greengard is nothing if not catholic in his enthusiasm for protein phosphorylation, and the actions of steroid and peptide hormones, insulin, interferon, all the neurotransmitters (excepting acetylcholine at nicotinic synapses) as well as the diverse effects of calcium ions are all envisaged by him as proceeding by this mechanism.

One's unease develops as the list increases; can the expression of all this biological diversity really be channelled through the simple phosphorylation of protein? It is clear that the regulation of glycolysis proceeds by cyclic AMP-induced protein phosphorylation. Other cases exist, but the argument from the particular to the general is not well founded. The mere correlation of varied cyclic nucleotides with protein phosphorylation in various circumstances is necessary but not sufficient to demonstrate it. It may be that protein phosphorylation is a side-effect of increased cyclic nucleotide levels rather than a mandatory stage in the expression of

their regulatory activity.

And even if protein phosphorylation was a mandatory stage, does this tell us much about the processes anyway? After all even if the theory were true, the important questions surely are; what proteins in each individual case are phosphorylated and how does this affect their physiological activity? Professor Greengard would probably agree that these questions are important and protest that he is merely proposing a working hypothesis for the detailed investigation of individual cases. But the trouble is that protein phosphorylation as a working hypothesis is so general and vague that it does not really help us formulate specific questions about regulatory mechanisms.

I turn now to the particular role of protein phosphorylation in neuronal and synaptic activity, Professor Greengard's special interest. He has proposed a scheme whereby a neurotransmitter (presumably the author has noradrenaline in mind, though it is not specified) is bound to its postsynaptic receptor and activates adenyl cyclase; this stimulates protein phosphorylation which causes a permeability change and thus a change in the membrane potential. Now, as Professor Greengard shows, the phosphorylative and enzymic activities prescribed by the scheme are found in the synaptic region but it is by no means clear that phosphorylation of the membrane protein causes the membrane conductance change that is known to be caused by transmitter action. The best experimental evidence that the author can offer is that isoproterenol (known to cause an increase in cyclic AMP) causes in turkey erythrocytes an increase in sodium influx, and protein phosphorylation, the dose- and time-response curves of which are similar. There are various correlations but none distinguishes between protein phosphorylation being a result of an increased ion flux rather

than its cause. I believe this to be crucial; but even if it were conceded it is a far cry from the permeability changes of the turkey erythrocyte membrane, measured in minutes, to that of the synapse measured in milliseconds. Indeed, the whole problem of timing is ignored by Professor Greengard. As it is proposed that the membrane conductance change results from the sequential action of two enzymes, it is surely incumbent on the author to show that the substrate concentration and enzyme activities are sufficient to enable protein phosphorylation to be fast enough to account for the speed of the permeability changes.

In another section Professor Greengard described his experiments in which calcium influx into synaptosomes causes a phosphorylation of a particular protein (protein I) that he has identi-

fied as being only present in the synaptic region. This is interesting, but he concludes (p77) "that calcium is a regulatory agent some of whose affects may be mediated by protein phosphorylation". It may; but these experiments have only demonstrated that protein phosphorylation is one of the many sequelae of depolarisation in the presence of calcium, which is not the same thing at all. And it is this type of reasoning which eventually alienates support from the author's general hypothesis; this is a pity, as the subject of protein phosphorylation, to which Professor Greengard has made distinguished contributions, deserves careful analysis without preconception as to its rôle.

R. M. Marchbanks

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Photosynthetic bacteria

The Photosynthetic Bacteria. Edited by R. K. Clayton and W. R. Sistrom. Pp. 946. (Plenum: New York and London, 1979.)

THIS very ambitious book attempts to provide an overall review of the present state of knowledge on all aspects of the study of photosynthetic bacteria at the highest level. It contains review articles by experts in many fields reflecting the importance of photosynthetic bacteria as excellent "simple" systems for studies of photosynthesis, membrane biogenesis, heme and carotenoid biosynthesis, and so on. This encyclopaedic approach has advantages and disadvantages. The main advantage of this book is that it provides an enormous amount of basic information about photosynthetic bacteria in readily accessible form. It must therefore become an essential reference work for any laboratory doing research on these organisms. However, the book has a number of disadvantages which arise from the attempt to be completely comprehensive. Almost every topic is covered by two chapters by different authors. This results in considerable duplication of material, even to the extent of printing the same electron micrograph in two chapters, and of course in conflicting interpretations. The result is to make the book much larger and more difficult to use than is required by the subject.

A more serious problem is that the book is out of date. It would seem that many, although not all, of the

chapters were completed in 1975, with minor updating to 1977. Although this is not too important for much of the background information, it is disastrous for articles dealing with rapidly moving research areas. The problem is made worse by variations in the amount of updating authors have done, and the confusion of multiple chapters dealing with each subject. For example, the chapter on primary electron acceptors does not mention the role of bacteriopheophytin in the reaction centre, because, as the author states, it was written in 1975 before this work was published. The work is in fact described in varying detail in three other chapters, although the chapter titles would not lead the uninitiated reader to look in them for this information.

The book is an excellent source of information on photosynthetic bacteria, but it must be used with a fairly critical attitude. It cannot therefore be recommended to non-specialists as a completely satisfactory dictionary of its subject.

M. C. W. Evans

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● An English language edition of *Einstein* by Louis de Broglie, Louis Armand, Pierre-Henri Simon *et al.*, originally published in French by Hachette (Paris) in 1966, has now been published by Peebles Press International (New York) and David and Charles (Newton Abbot, UK) at £7.50.

● *The Runaway Universe* by Paul Davies (for review, see *Nature*, 272, 785; 1978) has been published in paperback as *Stardoom* (Fontana: London), price 95p.

Capture-recapture methods

Investigating Animal Abundance. By M. Begon. Pp. 97. (Edward Arnold: London, 1979.) Paperback £3.95.

STUDYING the population dynamics or genetics of a natural population requires some knowledge of the changes in absolute population density over a period of time or between different locations. From this follows the opportunity to quantify the processes of birth and death, emigration and immigration. Adequate techniques for estimating population size are therefore of crucial importance to the study of natural populations.

Michael Begon has gathered together in a short volume a guide to a family of techniques for estimating absolute population size, all involving the mark, release and recapture of individuals from a population. These techniques (for example, Lincoln Index, Bailey's Triple Catch, Fisher-Ford Method, Jolly's Stochastic Method and Manly-Parr Method) have proved useful in the study of many populations from fruit flies, mosquitoes and grasshoppers to brown rats and salmonids. In the past, these capture-recapture methods have been a favourite hunting ground for statisticians who have delighted in the underlying theory. Begon is a biologist and has written for biologists, hoping to show "the essential simplicity of the mathematical techniques" and their potential as a biological weapon. In this he succeeds by virtue of a succinct prose, a clear lay-out and the good use of worked examples.

Capture-recapture methods are, however, only one of many techniques for estimating population size. This is very clear from T. R. E. Southwood's book, *Ecological Methods* (Chapman and Hall: London, second edition; hardback £10, paperback £3.75; for review, see *Nature* 278, 673; 1979), where over 50 pages are devoted to mark-release-recapture, but also nearly 100 pages to other methods for estimating absolute population size and a further 50 or so to techniques for relative population estimates. With Begon's aim of putting the biologist and his problems foremost, I would have liked to see, if only in passing, the subject viewed in its wider context.

Nevertheless, this book will be valuable for students who have to learn capture-recapture methods, for their teachers or for anyone contemplating their use in a research project.

M. P. Hassell

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One-dimensional conductors

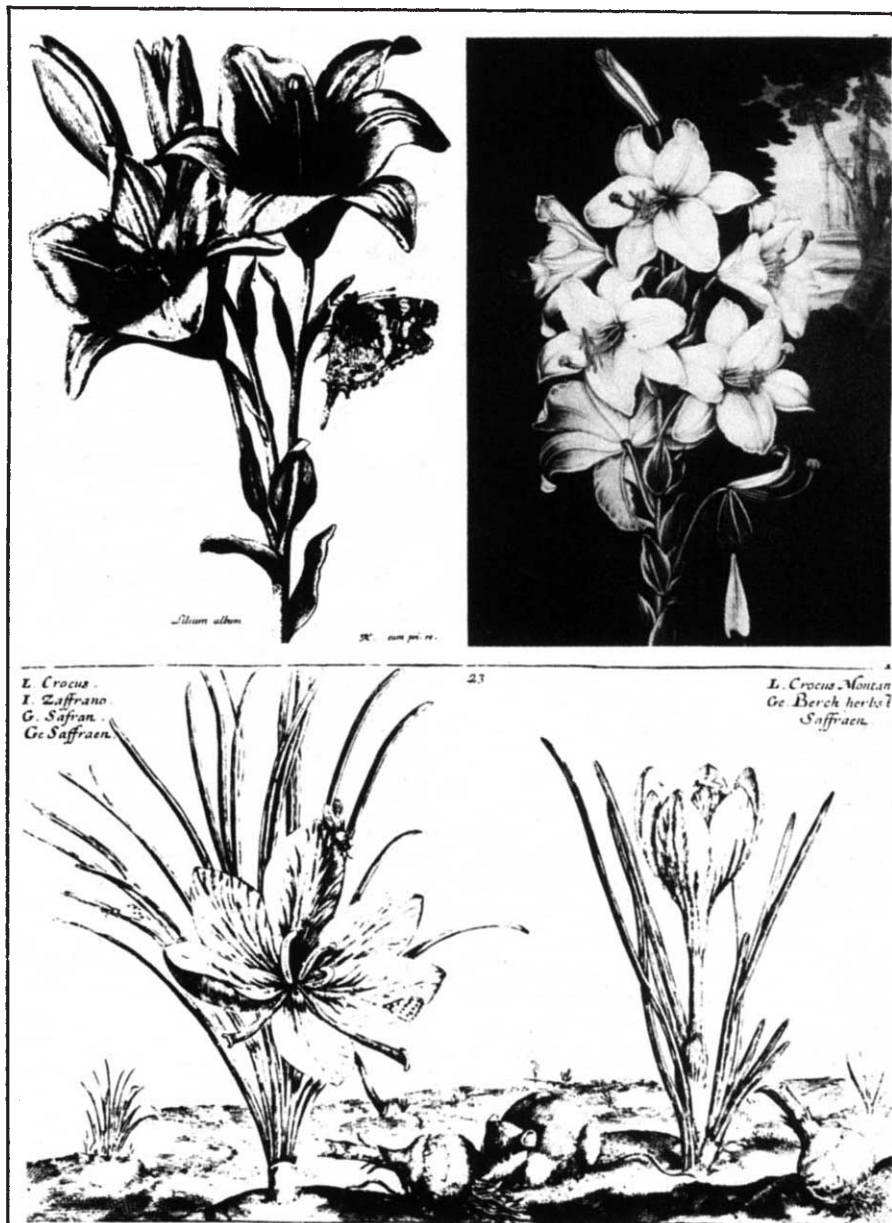
Highly Conducting One-Dimensional Solids. Edited by J. T. Devreese, R. P. Evrard and V. E. Van Doren. (Plenum: New York and London, 1979.) £26.77.

'ONE-DIMENSIONAL CONDUCTORS' are very popular at present because of the great diversity of physical phenomena which they exhibit. There are, of course, no truly one-dimensional conductors, and what we are dealing with are highly anisotropic solids composed on linear or chain-like structures. The systems which are discussed in some detail in this book are KCP or $K_2Pt(CN)_4 \cdot Br_{0.3} \cdot xH_2O$, and the organic charge transfer system TTF-TCNQ and its derivatives. In these solids the electronic anisotropy is the result of the directional nature of the orbitals involved in the formation of the conduction band. Mechanically, these two systems are almost isotropic. Other very anisotropic systems would include the $(SN)_x$ polymer, $NbSe_3$, and polyacetylene-halogen complexes.

One of the main interests in the study of these systems is the search for high electrical conductivity or high temperature superconductivity. A theoretical paper by Little in 1964, in which an electron-exciton interaction was proposed as a likely mechanism for high temperature superconductivity has been an underlying influence in this work. Following this, initial reports by the Pennsylvania group in 1973 on the high conductivity of TTF-TCNQ below room temperature, sparked off the present spate of activity. Since then, the observation of incommensurate superstructures, which have been interpreted as Peierls distortions, forms an important aspect of this work. The degree to which one dimensional fluctuations depress the Peierls transition to zero temperature, and the competition between electron-electron coulomb repulsion and electron-phonon coupling on the Peierls transition, provide some of the main interests which are discussed in great detail in this book.

This is the most coherent treatment which has so far been given on the subject. The book consists of eight chapters written by well known experts deeply involved in the subject. The chapters are by Berlinsky, Comes and Shirane, Heeger, Schultz and Craven, Sham, Emery, Gutfreund and Little, and Bardeen.

It is, of course, impossible to cover even the main points in a short review. The book leans heavily on the more physical aspects, and even here perhaps on the more theoretical problems. This



Top left, Madonna Lily, etching by Nicolas Robert, from his *Diverses Fleurs* (1675); top right, White Lily, aquatint by J. C. Stadler, in R. J. Thornton's *Temple of Flora* (1799-1807); bottom, Crocuses, line engraving by Crispin de Passe, from his *Hortus Floridus* (1614).

A new exhibition, entitled *Flowers in Art from East and West*, opened at the British Museum on 10 May and will continue until 9 September, 1979. The exhibition brings together a wide range of floral art, including the work of many of the greatest painters and draughtsmen working in many parts of Europe and Asia from the ninth century AD to the present day. The various techniques of European print-

making and the woodblock printing of the Far East are also illustrated. A book of the same title, from which the above illustrations are taken, is being published simultaneously by the British Museum Publications Department. Paul Hulton, Deputy Keeper of Prints and Drawings, and Lawrence Smith, Keeper of Oriental Antiquities, provide not an accompanying catalogue to the exhibition but a proper book which can be read on its own and which covers the development of floral art, starting with the early manuscripts of the Byzantine Empire. The book is beautifully produced, with both colour and black and white illustrations, in hardback (£7.95) and paperback (£3.95).

should not frighten off those interested in the more experimental or chemical side of this topic, as there is a good deal to learn from the contents. It is certainly the best treatment of the subject of one-dimensional systems that

I have seen, and it can be warmly recommended.

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obituary

Leland J. Haworth

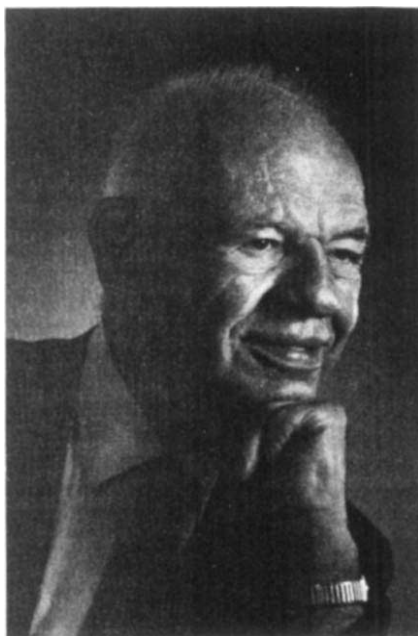
DR LELAND J. HAWORTH, prominent physicist, administrator and government official, died on 5 March 1979, in Port Jefferson, New York, at the age of 74. A former director of the Brookhaven National Laboratory, commissioner of the United States Atomic Energy Commission, and director of the United States National Science Foundation, he brought to those organisations a wealth of experience as a teacher, a research scientist, a project leader and a science administrator.

His activities were not restricted to science development in the U.S. only. A strong advocate of international co-operation in science, he played an active role in fostering such co-operation.

Leland Haworth first became involved with 'big science' when he was engaged in major microwave radar research and development programmes at the Massachusetts Institute of Technology's Radiation Laboratory from 1941 to 1946. His responsibilities grew from those of a components development engineer to those of head of the Receiver Components Division and a member of the laboratory's steering committee. His specialty was electronics, especially in data presentation and precision instrumentation.

Following a brief return to the University of Illinois as professor of physics, Haworth in 1947 joined the Brookhaven National Laboratory (BNL) as assistant director in charge of special projects. Brookhaven was established in 1946 by the United States Government and Associated Universities, Inc. (AUI) as a multidisciplinary research institution, a primary purpose of which was to provide special facilities essential for basic research in nuclear and related sciences.

Haworth was appointed director of Brookhaven in 1948. He stimulated the development of a truly user-oriented philosophy of service to the scientific community, including the development and provision of ever more advanced facilities. In the early 1950s the Cosmotron (a 3.0 GeV proton accelerator) and the graphite research reactor were brought into service. The 30 GeV alternating gradient synchrotron (AGS) was authorised in 1954, brought into operation in 1960, and today remains one of the key elements in the United States high energy physics programme. Similarly, under his



Brookhaven National Laboratory

direction, construction of the high flux beam reactor (40 MW) was initiated in 1959, and today it is still the major U.S. research reactor providing external high intensity beams of neutrons for research in physics, chemistry and biology.

Haworth's position as director of Brookhaven and his personal interest in high energy accelerator development were of great benefit to CERN both in the initial planning and development of that institution and in bringing the alternating gradient focusing concept, through CERN and BNL joint studies, to a reality in the Brookhaven AGS and the CERN proton synchrotron. This close cooperation between the two institutions has continued.

From 1951 to 1960, Haworth also served as vice president of Associated Universities, Inc. He was named president in 1960 and held that post, while continuing as laboratory director, until the spring of 1961.

Leland Haworth immersed himself completely in every activity he undertook. His drive, his insistence on facts and accuracy, his pursuit of excellence and his clarity of expression were hallmarks. If Haworth did it, it was done well. It might have taken longer but it was done correctly. His leadership and, above all, his consideration for staff and regard for their capabilities and achievements earned the respect and admiration of all of his associates.

Haworth's influence extended beyond Brookhaven: his services were in

demand as a member of numerous study groups and advisory panels—for the White House, the Department of Defense and other U.S. Government Agencies.

In 1961, President John F. Kennedy appointed Haworth commissioner of the U.S. Atomic Energy Commission. He rapidly became involved in matters of national security, civilian energy development and the support of fundamental research. As a commissioner, he was heavily involved in the nuclear weapons development programme and in the preparation of the 1962 AEC Report to the President on civilian nuclear power.

Haworth recognised the urgent need for restraint in nuclear weapons development, especially in the test programmes then carried out primarily in the atmosphere. He advocated a test ban that could be readily monitored by national means, an approach that led to the limited test ban treaty prohibiting testing in the atmosphere and under water.

The Atomic Energy Commission's 1962 Report to the President on civilian nuclear power was a masterpiece of its time. It was a major governmental energy planning document and, though its thrust was nuclear, reference was made to the need for conservation and to the development of coal resources. Haworth's analytical approach, rigorous logical presentations and other contributions to the study gave it substance and credibility and set the tone for what is now a necessary ingredient of all energy planning.

President Kennedy asked Haworth to leave the Atomic Energy Commission in order to become director of the National Science Foundation; he assumed office on 1 July, 1963. During Haworth's six-year term, he brought the foundation to maturity in a period that saw great expansion of federal support for the conduct of basic science. He guided the development of a programme which increased the number of high-quality institutions across the nation, and he pioneered NSF efforts to link fundamental science to applied science and technology.

Haworth returned to AUI in 1969 as special assistant to the president. He continued as a consultant to the president and to the BNL director following his retirement in 1977.

Leland John Haworth was born in Michigan on 11 July 1904. He grew

up in Indiana and attended Indiana University, receiving his AB in 1925 and AM in 1926, both in physics. In addition to his academic pursuits, he participated in varsity baseball and tennis. He received his PhD in physics from the University of Wisconsin in 1931.

He was an instructor in physics at the University of Wisconsin from 1930 to 1937. During his graduate work and until 1934, he did research in solid state physics, specialising in the surface structure of metals. In 1934 he switched to nuclear physics, which thereafter remained his principal field.

Following one year as a Lalor Fellow in physical chemistry at the Massachusetts Institute of Technology, Haworth, in 1938, joined the physics department of the University of Illinois. His association with Illinois continued until 1947 except for five years of war-time leave at MIT.

Dr Haworth was a member of the board of directors of Oak Ridge Associated Universities (ORAU) from 1959 to 1961, and again from 1971 to February 1978, when he was elected director emeritus—the first time ORAU had made such an appointment.

He was a member of the National Academy of Sciences, the American Philosophical Society, Phi Beta Kappa and Sigma Xi. He was a fellow of the American Physical Society, the New York Academy of Sciences, the American Academy of Arts and Sciences, and the American Nuclear Society (member of board of directors, 1955–60; president, 1957–58).

For his work during World War II, Haworth received the President's Certificate of Merit. A mesa in the Antarctic and an asteroid have been named in his honour. He was the recipient of many honorary degrees.

The name of Haworth is synonymous with meticulous care for the work at hand. His traits of objectivity, attention to detail and sensitivity to the problems of others influenced all who worked with him, and we are the better for it. The institutions with which he worked have been left with a Haworth heritage, and they are the better for it.

Gerald F. Tape

W. R. Aykroyd

DR WALLACE RUDELL AYKROYD, CBE, who died on 7 February 1979, aged 79, was a nutritionist of international renown since the early days of his career. His ability for meticulous work and great clarity of thought were soon recognised and his powers of organisation and administration left their mark in the many posts of distinction he held, particularly as the first Director of the Nutrition Division of the Food and Agriculture Organisation of the United

Nations from 1946 until 1960. His capacity to marshal facts and produce scientific reports in stylish and fluent prose made his publications important and influential in the fields of nutrition, public health and agriculture.

Graduating in medicine at Trinity College, Dublin, in 1924, where he was awarded the Vice-Chancellor's prize in English prose, he was appointed House Surgeon at the General Hospital, St John's, Newfoundland. He became interested in the deficiency diseases of the poorer fishermen and published an article on vitamin A deficiency in 1928 and a comprehensive report, 'Beriberi and other Deficiency Diseases in Newfoundland and Labrador', in the *Journal of Hygiene* in 1930. This 30-page report illustrates well the qualities which were to become the hallmark of his work—a broad approach, clear presentation of facts and a deep compassion for the unfortunate victims of malnutrition. He recognised that the diseases found were not due to deficiencies of single nutrients but multiple deficiencies due to poverty and a monotonous winter diet. His findings were used, and confirmed, by a team, which included B. S. Platt and W. H. Sebrell, conducting a medical survey of nutrition in Newfoundland some fifteen years later.

After his appointment to the Health Organisation of the League of Nations in 1931 he pursued his research into the link between malnutrition and poverty at a time of international recession and severe unemployment. His report, *Diet in Relation to Small Incomes*, published by the League of Nations in 1933, included a scholarly discussion on adequate dietary standards and satisfactory nutrition at low cost and his critical appraisal and distrust for the high protein allowances recommended was well in advance of his time and makes salutary reading today. The report is a valuable historical record of the time, containing studies of working class diet and expenditure on food in Germany, England and the USA.

The Health Organisation of the League of Nations decided, in 1934, that a general report on nutrition was needed after some ten years of studies of nutrition in the field of public health. Dr Aykroyd and Dr Etienne Burnet were asked to undertake this by making a series of enquiries in different countries and to report on nutritional status, research and policies. Their publication, *Nutrition and Public Health*, printed by the League of Nations in 1935, was an important statement of the current nutrition situation and was used extensively by international committees in public health and agriculture as well as the International Labour Office. Following this report the Health Organisa-

tion set up a Technical Commission for the Study of Nutrition which convened meetings of groups of experts who then produced reports (a procedure continued by FAO and WHO). The first report, *The Physiological Bases of Nutrition* (1936), relied heavily on the findings of the Aykroyd and Burnet report.

Following his appointment as Director of the Nutrition Research Laboratories in Coonoor, India, in 1935, Dr Aykroyd continued his work as an expert for the Technical Commission including research on the effect on nutrients of the milling of cereals and the influence of climate on food requirements. His work in India greatly enhanced the reputation of nutrition research in that country and the world famous Indian National Institute of Nutrition at Hyderabad was the result of his efforts. To this day, revised editions of *The Nutritive Value of Indian Foods* and *The Planning of Satisfactory Diets* retains his name as principal author in recognition of his original concepts for the work.

During his period at FAO from 1946 Dr Aykroyd's leadership gave the Nutrition Division a fine start and the reports of the Expert Committees (some jointly with WHO), which owed a lot to his guidance and editing, are consulted throughout the world. After his retirement from FAO, in 1960, he joined Professor B. S. Platt at the Department of Human Nutrition, London School of Hygiene and Tropical Medicine, where, as Senior Lecturer, he helped to set up regular courses which led to the present MSc in Human Nutrition, attended by graduates from all over the world. He wrote several books at this time including *Food for Man* and *The Conquest of Deficiency Diseases* which explained nutritional problems in terms understandable and interesting for the untrained reader. During his work as co-author of the FAO Studies, *Legumes in Human Nutrition* and *Wheat in Human Nutrition* he was able to indulge in his love of history as well as use his powers of marshalling a mass of facts into lucid and readable prose.

This interest in history, which had led to the writing in 1935 of *Three Philosophers*, one of whom had been his great hero, Lavoisier, was also evident in his book *Sweet Malefactor: Sugar, Slavery and Society*.

During his active retirement he carried on with nutrition consultancy work for WHO and OXFAM and found time to write *The Conquest of Famine*. A fitting tribute was made when this received the first award of a joint book award scheme of the British, Swedish and American Nutrition Foundations.

Joyce Doughty